ADVANCING WASTEWATER VALORIZATION USING ANAEROBIC MEMBRANE BIOREACTORS WITH BIOAUGMENTATION AND ION-EXCHANGE, AND BIO-PLASTIC PRODUCTION

BY

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DISSERTATION

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ABSTRACT

Wastewater treatment is essential for protecting human health and the environment. However, current conventional wastewater treatment, which focuses primarily on aerobic conversion of organic pollutants to CO_2, requires significant energy input making it costly and less environmentally sustainable. With increasing economic development, population growth, aging infrastructure, and stricter regulations, the energy and material inputs of wastewater treatment are only expected to increase (EPA, 2006; Mo & Zhang, 2013). Meanwhile, the carbon content of wastewater has potential to be a significant renewable resource for energy and materials production that could be leveraged to offset the cost and resource demands of wastewater treatment. Thus, shifting the current paradigm from pollutant removal to resource recovery is as a promising strategy for improving the economic and environmental impacts of wastewater treatment. To that aim, this work investigated two emerging technologies for resource recovery from wastewater, namely enhanced methane recovery in a novel two-phase anaerobic membrane bioreactor (AnMBR) process incorporating bioaugmentation and ion-exchange resins, as well as bio-polymer recovery via mixed microbial culture (MMC) polyhydroxyalkanoate (PHA) production.

The first study presented in this dissertation investigated the application of bioaugmentation in the acid-phase of a two-phase AnMBR treating primary sludge to improve solids removal and overall process efficiency. Bioaugmentation was carried out using a proprietary bioculture blend containing a mixture of hydrolytic, acidogenic, and acetogenic microorganisms. This mixture was added both on its own and in combination with recycled anaerobic sludge from the methane-phase of the AnMBR. These bioaugmentation strategies increased average percent hydrolysis by 25-38%, and increased average acid-phase acetic acid generation by 31-52% compared to operation without bioaugmentation. These benefits led to subsequent increases in average methane production (10-13%) and greater average overall solids reduction by 25-55%. Finally, microbial community analysis using 16S Illumina MiSeq generated sequences confirmed increased relative abundance of bioaugmented microorganism including *Acetobacter*, and *Syntrophomonas* species. Overall, bioaugmentation was found to improve conversion of primary sludge to methane by shifting the microbial community towards one better suited for hydrolysis and acetogenesis.

In the second study, application of ion-exchange resins in the methane-phase of the same two-phase AnMBR system was investigated as a means for improving reactor recovery after organic shock-loading. Four commercially available anion-exchange resins were evaluated for their ability to sorb soluble organics, specifically volatile fatty acids (VFA), from AnMBR effluent. The strong-base resin, Purolite TANEX was determined the best resin for deployment in the AnMBR system having achieved the greatest removal of soluble chemical oxygen demand (COD) (up to 36%) and acetic acid (up to 48%) in batch testing. Addition of 100 and 300 g/L of reactor volume of TANEX resin in a continuous flow AnMBR system improved effluent quality by reducing effluent COD concentrations by 48 and 75%, respectively. After shock-loading with 16,000 mg COD/L acetic acid, reactor recovery in terms of methane production was 9-58% faster with the addition of TANEX than without it. After shock-loading the system twice without the addition of TANEX, it was found that methane production recovery improved from 68 to 55 days, suggesting that acclimation of the microbial community also played a role in reactor recovery. Microbial community analysis using 16S Illumina MiSeq sequencing confirmed changes in the microbial community did occur as a result of shock-loading and the addition of TANEX resin. A higher average relative abundance of *Methanoscarcina* (up to 51 and 58%) was seen during operating periods with TANEX resin, leading to the conclusion that addition of the TANEX resin benefited reactor recovery by reducing stress on the microbial community via sorption of excess acetic acid, allowing the community time to adjust and become better able to process higher and more variable loadings of acetic acid.

In the third study, production of the biopolymer, polyhydroxyalkanoate (PHA), from hydrolyzed municipal organic waste was investigated as another approach to resource recovery from organic waste streams. The PHA production process was carried out in three phases beginning with (1) fermentation of the waste to produce a VFA-rich liquid effluent, (2) application of that VFA-rich fermentation liquid to select for PHA accumulating biomass, and (3) accumulation of PHA in the selected biomass using varying concentrations of the fermentation liquid to assess the effects of ammonium-nitrogen concentration on PHA accumulation. Preliminary batch testing to determine optimal operating parameters for the fermentation phase revealed that 5.4% solids content, 37°C, and 3.4 day retention time resulted in the greatest VFA production. Up to 14 g/L VFA production was achieved in lab-scale continuous fermentation of municipal organic waste. The liquid fraction of the fermented

material was applied using a feast/famine feed strategy to successfully select PHA accumulating biomass. Finally, the PHA accumulation phase achieved an average maximum yield of 38% PHA/g VSS using a low ammonium-nitrogen feed mixture. Application of clinoptilolite was determined to be an effective means for reducing ammonium-nitrogen concentration in the fermentation liquid and improved PHA accumulation by up to 29%. Overall, this study demonstrated the feasibility of using a complex organic waste stream, namely municipal organic waste, for mixed microbial culture PHA production, with the potential for nutrient recovery as well.

Finally, life cycle assessment methodology was applied to evaluate and compare the environmental impacts associated with the two resource recovery options, i.e. methane recovery via AnMBR treatment, and bio-polymer recovery via MMC PHA production, considering primary sewage sludge as the substrate. Overall, the AnMBR process was determined to be the more environmentally sustainable option achieving a reduced environmental impact in 6 out of the 10 impact categories considered. Energy consumption was determined to be the largest contributor to overall environmental impact for both processes. However, in the case of AnMBR treatment, it was estimated that more than enough energy could be recovered as methane to offset energy requirements and achieve a positive energy balance. In the case of PHA production, the high energy requirements for aeration negatively impacted the global warming potential (GWP) of the PHA process, although it performed better in the impact categories of fossil fuel depletion and ecotoxicity compared to the AnMBR process. Uncertainty and sensitivity analysis suggested that, under optimized conditions, it may be possible to achieve a net negative GWP for PHA production from primary sludge. In addition, an initial economic assessment that included only operating input costs and potential revenue from recovered methane and PHA products suggested that the relatively high selling price of PHA could more than offset the operating input costs for its production, potentially leading to greater economic benefits compared to the AnMBR process. In the end, a combination of the two technologies may be an advantageous option for improving the environmental and economic sustainability of wastewater treatment. However, a more detailed techno-economic analysis, including consideration of capital costs and PHA extraction is needed. In addition, LCA predictions should be validated with large-scale, long-term demonstration of the two technologies.

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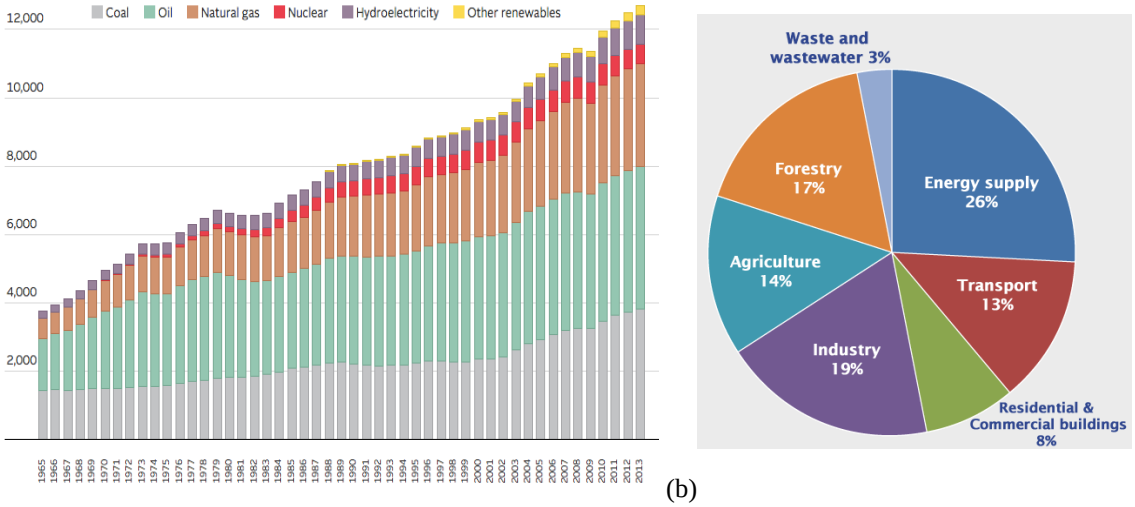
CHAPTER 1: INTRODUCTION

Background

Over the past decades, global economic development and population growth have contributed to increased global energy consumption (Yang et al., 2015). At the same time, the burning of fossil fuels, including coal, oil, and natural gas, which currently account for more than 80% of the global energy supply (World Bank, 2014), is widely recognized as the largest contributor to global greenhouse gas (GHG) emissions (Schilling, 2013). Figure 1.1a shows the increase in global energy demand from 1965 to 2013 and Figure 1.1b shows the breakdown of global GHG emission by source. Increasing GHG concentrations in our atmosphere are in turn leading to unprecedented increases in global temperature and climate change, exacerbating issues related to global water scarcity, food security, and ecosystem functioning (NASA, 2017; IPCC, 2014). Therefore, there is a critical need to reduce global GHG emissions and minimize our consumption of fossil fuel resources by utilizing alternative renewable resources for energy and materials production.

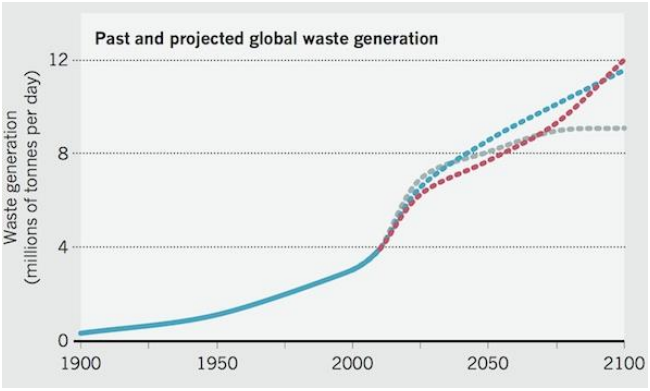


(a) (b)

Figure 1.1. (a) Global energy use by source (Plumer, 2015), and (b) breakdown of GHG emissions by sector (Schilling, 2013).

In addition to energy consumption, global waste production has also increased over the past decades, contributing to approximately 3% of global GHG emissions as shown in Figure 1.1b (Schilling, 2013). Figure 1.2a shows global municipal solid waste generation over time. The majority of waste being generated, including municipal solid waste, municipal wastewater,

agricultural residues and manures, food and other industrial waste, contains a large fraction of organic material. Approximately 46% of total global waste is organic waste, as shown in Figure 1.2b (Yang et al., 2015). Thus, organic waste represents a significant renewable resource that could be leveraged for energy and biomaterials production. Additionally, utilizing organic waste for resource recovery provides the duel benefit of reducing the use of fossil fuel resources, and eliminating wastes that may otherwise be a hazard to human health and the environment.

(a) 

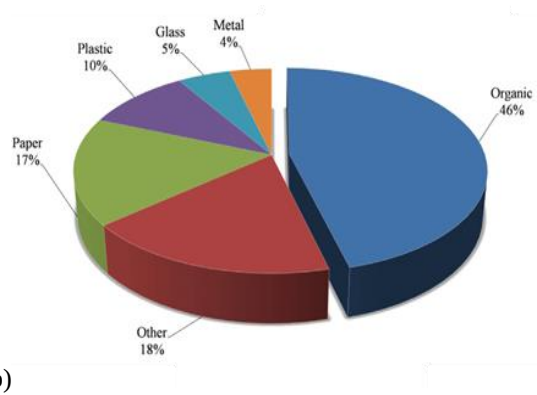
(b) 

Figure 1.2. (a) Global waste production from 1900 to 2013 and projected generation from 2013 to 2100. (Stromberg, 2013), and (b) average global solid waste composition (Yang et al., 2015).

In municipal wastewater treatment, researchers and operators are recognizing the need to treat wastewater not as waste but as a resource for water, energy, and nutrient recovery, while at the same time reducing the impact of treatment processes on GHG emissions (Sutton, et al. 2011). As a result, application of anaerobic treatment processes in mainline wastewater treatment is gaining increasing attention due to its many advantages over conventional aerobic treatment. Current conventional wastewater treatment relies on aerobic degradation of organics to carbon dioxide. The energy required to supply oxygen in these processes accounts for approximately 55% of the total energy demand of the treatment plant, or approximately 0.65 kWh/m^3 of treated wastewater (McAdam, 2010). In the U.S., wastewater treatment accounts for an estimated 3% of the total U.S. electricity demand (EPA, 2006). In contrast to aerobic treatment, anaerobic treatment offers the advantages of energy recovery as methane-rich biogas, as well as reduced energy demand since it does not require aeration, and reduced waste sludge production (Chang, 2014; Dvořák et al., 2016; Lin, et al., 2013). Despite these many benefits, anaerobic treatment is currently limitedly applied in wastewater treatment due to several factors including longer

retention times compare to aerobic treatment, which lead to high capital costs and large land requirements in order to accommodate large reactor volumes. In addition, compared to aerobic processes, anaerobic processes usually require longer start-up, result in poorer effluent quality, and suffer from greater process instability. In order to address these challenges, the development of anaerobic membrane bioreactors (AnMBR), which combined anaerobic treatment with membrane filtration, has become a growing area of research. By decoupling solid and hydraulic retention times, AnMBRs can reduce required reactor volumes, improve solids removal, increase methane production, improve process stability, and provide a solids free, nutrient-rich effluent for potential reuse. However, there are several challenges regarding AnMBRs that still need to be overcome, primarily relating to solids accumulation, membrane fouling, and low temperature performance.

An alternative to energy recovery is the production of biomaterials from organic waste. One such material is polyhydroxayalkanote (PHA), a biodegradable, biopolymer that can be used as an alternative to petroleum based polymers for the production of plastic materials. Similar to anaerobic treatment for methane recovery, PHA production utilizes the carbon content of the waste, beginning with fermentation of organic material into volatile fatty acids (VFA). The resulting VFA-rich fermentation liquid is then used as the feedstock for cultivation of PHA accumulating microorganisms. Presently, industrial processes for PHA production are based on the use of pure cultures of selected microbial strains that requires use of single, pure substrates for cultivation (Setiadi et al., 2015, Villano et al., 2014). This makes PHA production expensive and uncompetitive with synthetic thermoplastics, due to the costs of culture maintenance, substrate formulation, and both substrate and reactor sterilization (Ivanov et al., 2015). The current PHA price, ranges from $2.3-5.3/kg, compared to $1/kg or less for conventional petroleum-based polymers (Valentino, et al. 2017). Therefore, in order for PHA it to be able to compete with conventional petroleum based plastics, production costs need to be reduced. This has motivated research on the use of waste feedstocks and mixed microbial cultures (MMC) for PHA production. However, PHA production from complex waste streams is still a challenge, due to the inherent variability of the wastes, and generally high nutrient content. Therefore, further research is needed to demonstrate feasibility and maximize efficiency of PHA production from complex organic wastes.

This work investigated both AnMBR technology and MMC PHA production as methods for resource recovery from organic waste. Organization of the presented work is as follows: in Chapter 2, a literature review is presented in which the details of anaerobic treatment, AnMBRs, and MMC PHA production processes are discussed, as well as current challenges and potential solutions associated with these processes. In the subsequent chapters, Chapter 3 - 6, four different research studies are presented that investigate different aspects of the proposed AnMBR and PHA treatment processes. The specific research objectives for each of the four research studies are outline in the following section. Finally, the main conclusions and future work recommendations resulting from this work are summarized in Chapter 7.

Research Objectives

The first aim of this research was to develop and demonstrate a novel anaerobic treatment system that could improve the energy balance for wastewater treatment and enhance opportunities for water reuse. Such a system could be employed in many sectors of society that create and/or process organic laden wastewaters including municipal, agriculture, industrial, and military (e.g. military bases) sectors. The major limitations to increasing the use of anaerobic systems are longer retention times, instability/sensitivity of anaerobic microbial communities, and insufficient effluent water quality. In this research, a two-phase anaerobic membrane bioreactor (AnMBR) system was developed to address these items, incorporating bioaugmentation with hydrolytic and acidogenic microorganisms as well as addition of ion-exchange resin to treat a high strength domestic wastewater. Figure 1.3 illustrates the proposed two-phase AnMBR system. The impacts of bioaugmentation and ion-exchange resin application in the two-phase AnMBR system were investigated in Chapter 3 and Chapter 4, respectively.

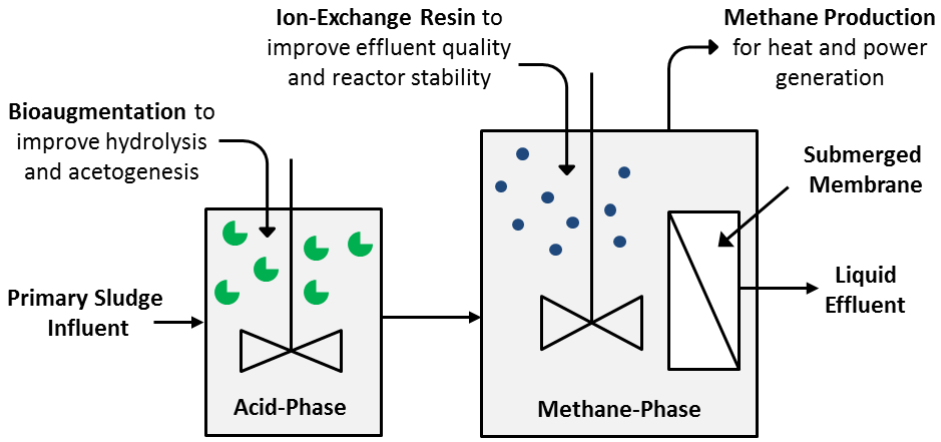


Figure 1.3. Schematic of proposed two-phase anaerobic membrane bioreactor system incorporating bioaugmentation and ion-exchange resin to improve process performance.

Chapter 3 Objectives:

Using the proposed two-phase AnMBR system, the first objective of this research was to investigate bioaugmentation as a means for targeting and improving hydrolysis and acetic acid production from primary sewage sludge at mesophilic temperature (37°C). It was hypothesized that the addition of a mixture of hydrolytic, acidogenic, and acetogenic microorganisms would shift the microbial population towards one better suit for hydrolysis and acetogenesis, leading to increased conversion of particulate organics to acetate, a precursor for methane, subsequently leading to increased methane production and overall solids removal.

Chapter 4 Objectives:

The second objective of this work was to determine whether the addition of an anion-exchange resin in the hydroxide form could improve process performance under ambient temperature conditions (20°C) and reactor recovery after organic shock-loading. It was hypothesized that exchange of hydroxide ion for negatively charge VFAs would help mitigate the pH change associated with organic-shock loading. In addition, it was hypothesized that sorption of excess VFAs onto the ion-exchange resin would reduce the loss of soluble organics in the effluent and lead to potential improvements in methane production.

Chapter 5 Objectives:

A second aim of this research was to demonstrate the feasibility of using complex waste streams as the feedstock for PHA production in a three-phase process including (1) fermentation of the organic waste to produce VFAs, (2) selection of PHA-accumulating biomass via a feast/famine feeding regime using the VFA-rich fermentation effluent as the substrate, and (3) PHA-accumulation. The three-phase process is shown in Figure 1.4.

The specific objectives of this study were to investigate the feasibility of using the organic fraction of municipal solid waste (OFMSW) as a feedstock for MMC PHA production. It was hypothesized that fermentation of OFMSW could provide a suitable VFA-rich substrate for selection of PHA accumulating microorganisms and subsequent PHA-accumulation, if applied in conjunction with ammonium-nitrogen removal. Therefore, additional objectives were to assess the impact of ammonium-nitrogen concentration on PHA accumulation, and to evaluate the effectiveness of the natural zeolite, clinoptilolite, as a means for removing/recovering ammonium-nitrogen from the fermentation liquid.

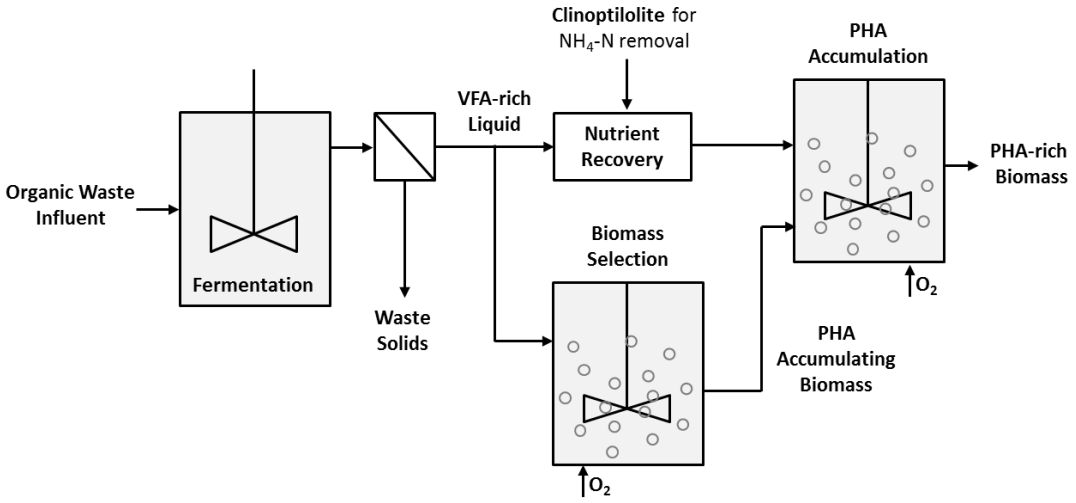


Figure 1.4. Three-phase process for MMC PHA production from organic waste with potential for nutrient recovery.

Chapter 6 Objectives:

Finally a life cycle assessment (LCA) was conducted with the objective of quantifying and comparing the potential environmental impacts of the two resource recovery processes, i.e. methane recovery via AnMBR and bio-polymer recovery via PHA production, considering primary sewage sludge as the substrate.

References

1. Chang, S. (2014). Anaerobic Membrane Bioreactors (AnMBR) for Wastewater Treatment. *Advances in Chemical Engineering and Science*, *4*(1), 56–61.

2. De Vrieze, J., Hennebel, T., Boon, N., & Verstraete, W. (2012). Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. *Bioresource Technology*, *112*:1–9.

3. Dvořák, Lukáš, Marcel Gómez, Jan Dolina, and A. Č. (2016). Anaerobic membrane bioreactors—a mini review with emphasis on industrial wastewater treatment: applications, limitations and perspectives. *Desalination and Water Treatment*, 1–15.

4. IPCC. (2014). *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. *Climate Change 2014: Synthesis Report*. Geneva, Switzerland.

5. Lin, H., Peng, W., Zhang, M., Chen, J., Hong, H., & Zhang, Y. (2013). A review on anaerobic membrane bioreactors: Applications, membrane fouling and future perspectives. *Desalination*, *314*, 169–188.

6. McAdam, E. (2010). Anaerobic workout. *The Chemical Engineer*. 833:38-39

7. Plumer, B. (2015). Global Energy Use by Source. Clean Energy is Growing Fast – but it´s not winning the race against fossil fuels. Vox. www.vox.com

8. Schilling, D. R. (2013). Global Greenhouse Gas Emissions by Source. The Big Picture Breakdown of Greenhouse Gases. www.industrytap.com

9. Stromberg, J. (2013). When will we hit peak oil?. www.smithsonian.com

10. The World Bank. (2014) Fossil fuel energy consumption. IEA Statistics. data.worldbank.org

11. U.S. Environmental Protection Agency. (2006). Wastewater Management Fact Sheet: Energy Conservation.

12. Yang, X., Choia, H. S., Parkb, C., Kima, S. W. (2015). Current states and prospects of organic waste utilization for biorefineries. *Renewable and Sustainable Energy Reviews*. 49:335–349

CHAPTER 2: LITERATURE REVIEW

Anaerobic Treatment: Process and Challenges

Anaerobic wastewater treatment is gaining increasing attention due to its many advantages over conventional aerobic treatment including the opportunity for energy recovery, as well as reduced energy demand, greenhouse gas emissions, and waste sludge production (Chang, 2014; Dvořák et al., 2016; Lin, et al., 2013; Ketheesan & Stuckey, 2015). Currently, anaerobic digestion is commonly applied as a side-stream process for stabilizing waste sewage sludge produced during conventional wastewater treatment (Liao et al., 2006; Pierkiel & Lanting, 2005). In this process, organic material is biodegraded via the concerted metabolic actions of a consortium of microorganisms in the absence of oxygen to produce a mixture of methane and carbon dioxide called biogas. The resulting methane-rich biogas can be used as an alternative to natural gas for heat and power generation.

The anaerobic digestion process is considered to consist of four sequential phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, through which complex particulate organic material is broken down into simpler soluble compounds, primarily volatile fatty acid (VFA) intermediates, which are ultimately converted into methane and carbon dioxide. Figure 2.1 outlines the four phases of anaerobic digestion and the pathways by which particulate organic matter is converted to methane and carbon dioxide. Each phase is carried out by a different group of microorganisms, namely hydrolytic, acidogenic, and acetogenic bacteria, and methanogenic archaea, which exist in syntrophy with one another, but differ in terms of their growth kinetics and optimal environment requirements. Two-phase anaerobic digestion offers a means for optimizing reactor conditions to accommodate the different groups of microorganisms, by physically separating the process into two reactors. In this case, the four phases of the digestion process are broadly grouped into two phases namely the “acid-phase” in which hydrolysis, acidogenesis and acetogenesis are typically carried out to produce a VFA rich effluent, and the “methane-phase” in which further acetogenesis and methanogenesis is carried out to convert acid-phase VFAs into methane and carbon dioxide. Each of the four phases of the anaerobic digestion process is described in further detail in the following sections. Two-phase digestion as well as some of the current challenges and limitations of anaerobic treatment are also discussed.

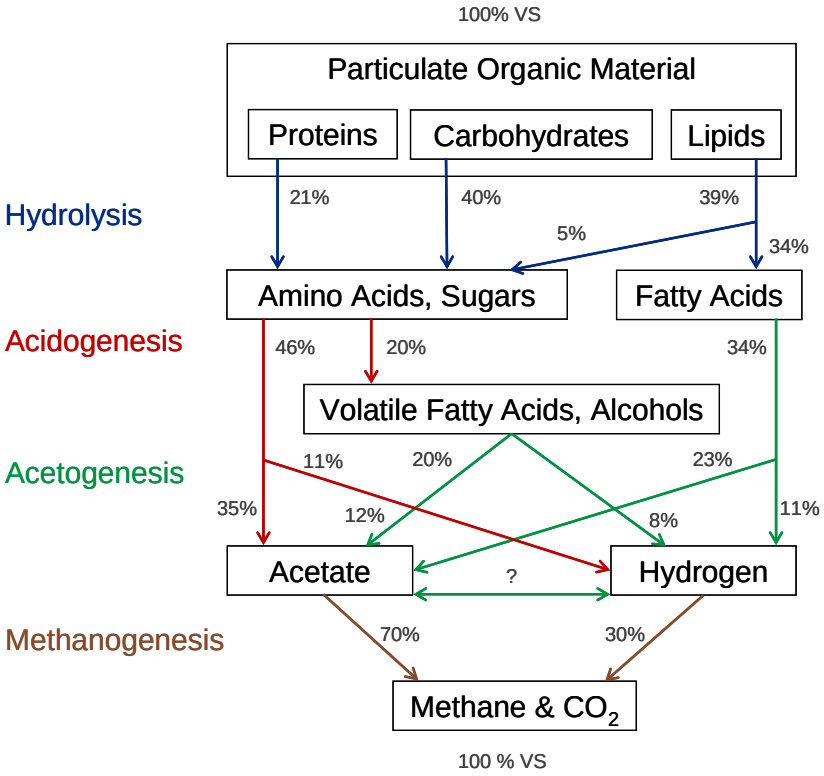


Figure 2.1: Phases and conversion pathways in anaerobic digestion (adapted from Gujer & Zehnder, 1983).

Phases of Anaerobic Treatment

Hydrolysis

The first phase of anaerobic digestion is hydrolysis. In this phase, complex particulate organic matter is broken down into smaller water soluble compounds, which can be taken up by microbial cells. Complex macromolecules including carbohydrates, proteins, and fats, are converted into monosaccharides, amino acids, and fatty acids respectively. This occurs via enzymatic hydrolysis, in which various facultative and/or obligate anaerobic hydrolytic bacteria excrete extracellular enzymes which facilitate the splitting of covalent bonds within the substrate in a chemical reaction with water (Chandra & Takeuchi et al., 2012; Shah et al., 2014). The enzymes involved in hydrolysis are called hydrolases. Different hydrolases produced by specific species of hydrolytic bacteria are required for degrading different macromolecules. For example cellulolytic bacteria produce cellulases for the hydrolysis of cellulose, while lipolytic bacteria produce lipases for the hydrolysis of lipid molecules. The rate of hydrolysis is dependent on a number of factors including pH, temperature, concentration of hydrolyzing biomass, and type

and size of particulate material (Shah et al., 2014; Visvanathan & Abeynayaka, 2012). Hydrolysis of non-structural carbohydrates occurs relatively quickly, on the order of a few hours, while hydrolysis of proteins and lipids can take up to a few days. Structural carbohydrates, including cellulose and hemicellulose are the most difficult to hydrolyze, and conversion of these molecules tends to be extremely slow and incomplete (Chandra, Takeuchi et al. 2012). It is estimated that during solid wastes degradation only 50% of organic compounds undergo compete biodegradation due to the lack of enzymes participating in their degradation (Shah et al., 2014). Therefore, methods for improving hydrolysis are important, particularly for waste streams with high concentrations of particulate solids, including sewage sludge, where hydrolysis can be especially limiting.

Acidogenesis

The second phase of anaerobic digestion is acidogenesis. In this phase soluble sugars, amino acids, and fatty acids produced in the hydrolysis phase are taken up by various acid-forming bacteria (acidogens) and converted into short-chain VFAs (e.g. formic, butyric, propionic, and acetic acid), as well as alcohols, aldehydes, hydrogen, and carbon dioxide. The products formed in this phase vary depending on the bacteria present and environmental conditions. Often times the same bacteria can perform both hydrolysis and acidogenesis. The acidogenic bacterial community may include facultative and/or obligate anaerobic bacteria. Examples include *Pseudoomonas, Clostridium, Bacillus, Micrococcus,* and *Flavobacterium* (Shah et al., 2014). In general acidogenic bacteria are relatively fast growing microorganisms and relatively tolerant to environmental changes (Visvanathan & Abeynayaka, 2012). This can present a potential problem if acidogens are able to grow and generate VFAs faster than they can be converted to methane. Acidic conditions are toxic to methanogens, therefore accumulation of VFAs will likely cause inhibition of methanogenesis and potentially lead to reactor failure.

Acetogenesis

The third phase of anaerobic digestion is acetogenesis. In this phase, VFAs, alcohols, and hydrogen produced in the acidogenesis phase are converted to acetate via acetogenic bacteria. Acetate is a key substrate for methanogenesis with 65-70% of methane production in anaerobic digestion processes being produced from the reduction of acetate (Ketheesan & Stuckey, 2015; Shah et al., 2014). Two groups of acetogenic bacteria play a role in acetogenesis. Obligate H_2-

producing acetogenic bacteria oxidize propionate, butyrate, and long-chain fatty acids to acetate, while homoacetogens consume hydrogen and carbon dioxide to produce acetate (Ketheesan & Stuckey, 2015). Under heterotrophic growth, homoacetogens can also consume a wide variety of other carbon substrates such as sugars and alcohols, generating acetate and hydrogen as end products (Diekert & Wohlfarth, 1994). The metabolic diversity if acetogens makes them tolerant to a wide range of environmental conditions (Zaher et al., 2007). Hydrogen plays an important role in acetogenesis, as the conversion of acidogenesis products, i.e. propionic acid, butyric acid, and alcohols, to acetate will only take place if the hydrogen partial pressure is low enough to thermodynamically favor the reaction. Thus, the presence of hydrogen scavengers, which include homoacetogens and hydrogenotrophic methanogens is an important factor in the acetogenesis phase. Table 2.1 summarizes a few acetogenic reactions and their associated Gibbs free energy change. Examples of acetogenic bacteria included several species from the genera *Clostridium*, *Acetobacterium*, and *Sporomusa* (Muller & Frerichs, 2013).

Table 2.1. Acetogenesis, methanogenesis and sulfate-reducing reactions (adapted from Stams et al., 2005).

Reaction Type	ΔG^0 (kJ/reaction)
Acetogenic	
$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + HCO_3^- + H^+ + 3H_2$	+76.1
$CH_3CH_2COO^- + 2H_2O + 2CO_2 \rightarrow CH_3COO^- + 3HCO_2^- + 3H^+$	+65.3
$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2\,CH_3COO^- + H^+ + 2H_2$	+48.3
Homoacetogenic	
$4H_2 + 2HCO_3^- + H^+ \rightarrow CH_3COO^- + 4H_2O$	-104.6
Methanogenic	
$4H_2 + HCO_3^- + H^+ \rightarrow CH_4 + 3H_2O$	-135.6
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	-31.0
Sulfate-reducing	
$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$	-151.9
$4CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^-$	-47.6
$4CH_3CH_2COO^- + 3SO_4^{2-} \rightarrow 4CH_3COO^- + 4HCO_3^- + 3HS^- + H^+$	-37.7
$2CH_3CH_2CH_2COO^- + SO_4^{2-} \rightarrow 4CH_3COO^- + HS^- + H^+$	-27.8

Methanogenesis

The last phase of anaerobic digestion is methanogenesis. In this phase, methane is formed under strictly anaerobic conditions via various species of methanogenic archaea. There are two major pathways for methane formation in anaerobic digestion. The primary route is conversion of acetate to methane and carbon dioxide. This is carried out by acetoclastic methanogens. Despite the fact that there are only two known genera of aceticlastic methanogens, *Methanosarcina* and *Methanosaeta*, (Ketheesan & Stuckey, 2015; Venkiteshwaran et al., 2016), as mentioned previously, approximately 70% of methane in anaerobic digestion processes is generated via aceticlastic methanogenesis. The other 30% is generated via hydrogenotrophic methanogens, in which hydrogen is used to reduce carbon dioxide to methane (Zaher et al., 2007). *Methanosarcina* sp. are able to use both aceticlastic and hydrogenotrophic pathways (Shah et al., 2014). Methanogenesis can often be rate limiting due to the slow growth rates of methanogenic archaea and their lower tolerance to inhibitory and toxic compounds such as ammonia. Slow growth rates make methanogens prone to wash-out. In addition, as previously discussed, if methanogens are not able to keep pace with faster growing acidogens and acetogens, VFA accumulation can also lead to process inhibition and potential reactor failure (Visvanathan & Abeynayaka, 2012). Finally, methanogenesis can also be inhibited by sulfate concentrations and competition between sulfate-reducing bacteria and aceticlastic methanogens since acetate oxidation via sulfate reduction is more thermodynamically favorable than methanogenesis (Table 2.1). Khanal and Huang (2005) noted that dissolved sulfide concentrations of 228 - 613 mg/L of free sulfide could impose toxicity on methanogens.

Two-phase Anaerobic Treatment

In conventional anaerobic treatment processes, a single reactor is used in which all four phases of the process take place. In this situation, because the hydrolytic and acid-forming bacteria differ from methanogens in terms of their environmental conditions, growth kinetics and sensitivity, a delicate balance must be maintained within the reactor in order to in avoid system failure. With that, conventional single-phase operation can be prone to upsets. Problems with stability and control in single-phase digestion have motivated research in the area of two-phase anaerobic digestion. Two-phase anaerobic treatment offers a method for optimizing the operating conditions for the various groups of microorganisms involved in the digestion process. In two-

phase digestion, the process is physically separated into two reactors. The first reactor is operated under optimal conditions for hydrolysis and acidogenesis and is referred to as the acid-phase reactor, while the second reactor is operated under optimal conditions for methanogenesis and is referred to as the methane-phase reactor. In this case, pH conditions can be maintained at appropriate levels in either reactor. Acidogens and acetogens can tolerate a relatively low pH, however methanogens are more sensitive, with an optimal pH range of 6.8-7.5 (De Vrieze et al., 2012). Two-phase digestion can also increase process stability by optimizing the retention time for either phase of the process. Typically, retention times are shorter in the acid-phase and longer in the methane-phase to accommodate for the variation in growth rate between the rapidly regenerating acidogenic bacteria and slow growing methanogens. Finally, two-phase operation allows for the selection and enrichment of different bacteria in each phase (Demirer & Chen, 2005).

Several studies have demonstrated the advantages of two-phase digestion over single-phase digestion. In general, two-phase digestion has been successful in treating a wide range of substrates including, but not limited to, domestic and industrial wastewaters (Van Lier et al., 1997; Ghosh, 1985; Ng, 1985; Yushina & Hasegawa, 1994; Gharsallah, 1994; Massey, 1978), municipal sewage sludge (Bhattacharya, 1996; Ghosh, 1987; Kugel et al, 1992), food processing wastes (Cohen et al., 1994; Raynal et al., 1998) the organic fraction of municipal solid waste (Cecchi et al., 1994; Pavan et al., 2000), forest residues (Hooper & Li, 1996) and wood hydrolysate (Chakrabarti et al., 1999). Zhang et al. (1991) compared single- and two-phase processes in terms of bacterial population levels and observed the number of acetate-utilizing methanogens was 2-10 times higher in the two-phase system than in the single-phase system. In two-phase digestion of soft-drink waste, Ghosh (1987) were able to achieve higher methane production and COD removal at lower HRT and higher loadings compared to conventional single-phase digestion. Similarly, Yeoh (1997) observed a threefold increase in methane yield from two-phase digestion of cane-molasses alcohol stillage compared to single-phase digestion. In general, Ghosh (1985) reported that two-phase anaerobic digestion of municipal sewage sludge resulted in higher efficiencies and conversion rates compared to conventional single-stage treatment at both mesophilic and thermophilic temperatures as well as at a variety of HRTs, loading rates, and feed concentrations.

Challenges in Anaerobic Treatment Processes

One of the major drawbacks associated with anaerobic treatment of wastewaters is the fact that anaerobic degradation of particulate organic material typically requires much longer retention times compare to aerobic treatment, on the order of 30-50 days (Chong et al., 2012; Yadvika et al., 2004). As a result, large reactor volumes are needed in order to accommodate such long retention times, which leads to high capital costs and large land requirements. For anaerobic treatment of waste streams with a high concentration of particular organics, such as municipal wastewater, sewage sludge, and municipal solid waste, hydrolysis has been recognized as the major-rate limiting step (Eastman & Ferguson, 1981; Teo & Wong, 2014, Mumme et al., 2010; Park et al., 2005). Thus, application of strategies for targeting and improving hydrolysis can be a promising means for improving anaerobic treatment of high solids content waste streams.

In addition, compared to aerobic processes, anaerobic processes usually require longer start-up times, result in poorer effluent quality, and suffer from greater process instability due to the slow growth rate of anaerobic microorganisms and their sensitivity to different environmental factors (De Vrieze et al., 2012). As evident from earlier discussion, anaerobic processes rely on a delicate balance among the various groups of microorganisms participating in order to ensure optimum conversion of influent organics to methane and avoid process failure (Ketheesan & Stuckey, 2015). A number of factors can upset the process stability of anaerobic systems including organic loading rate (OLR), solid retention time (SRT), pH, temperature, and toxicants (Ketheesan & Stuckey, 2015). Fluctuations in OLR is of particular concern for wastewater treatment. Sudden or unexpected increases in OLR can lead to accumulation of VFAs due to the slower growth rate of methanogens relative to acidogens and acetogens (Ketheesan & Stuckey, 2015; De Vrieze et al., 2012). VFA accumulation in turn reduces pH which can inhibit methanogenic activity. Therefore, in order to avoid overloading, conventional anaerobic treatment processes are typically operated below their optimum capacity (De Vrieze et al., 2012). In short, methods for improving process stability and protecting against/mitigating the effects of organic loading fluctuation and VFA accumulation are also needed for improving anaerobic treatment processes.

Anaerobic Membrane Bioreactors

Anaerobic membrane bioreactors (AnMBRs) offer a solution for the issues of long retention times, process instability, and poor effluent quality, associated with conventional anaerobic treatment. AnMBRs combine anaerobic treatment with ultra- or micro-filtration membrane technology to effectively decouple the hydraulic and solid retention times (HRT and SRT). The benefit of decoupling HRT and SRT is that the liquid fraction of the waste stream is able to pass through the system more quickly, reducing volume requirements, while the solids fraction, including waste solids and microbial biomass, is retained by the membrane providing sufficient time for degradation and conversion to methane. Figure 2.2 illustrates solid and liquid separation in an AnMBR. Longer SRTs are especially important for waste streams that contain particulate organic material, which require extended periods of time to degrade, and for ambient or low temperature treatment, as microbial activity and degradation rates decrease with decreasing temperature (Stuckey, 2012). Thus, besides reducing reactor volume, retention of solids by the membrane can lead to increased solids removal, methane production, and process stability, as well as production of a solids free, nutrient-rich effluent with potential for water reuse and/or nutrient recovery (Chang, 2014; Lin et al., 2013; Stuckey, 2012). Overall, AnMBRs offers many potential benefits in terms of resource recovery from wastewater, however further research and large-scale demonstrations are still needed in order to overcome current challenges, and validate the viability and sustainability of AnMBRs in municipal wastewater treatment contexts.

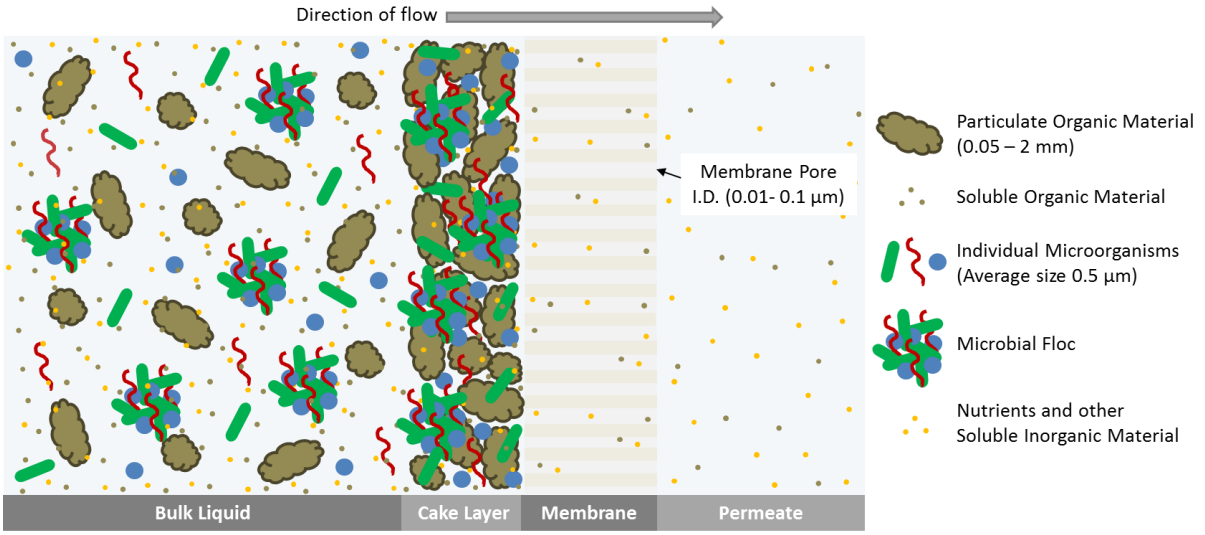


Figure 2.2. Illustration of AnMBR solid and liquid separation.

Current Status of AnMBR Technology

While the AnMBR is still an emerging technology, the concept of AnMBRs was first reported by Grethlein in 1978 in which an external cross-flow membrane was used to treat septic tank effluent (Lin et al., 2103). Since then, with the development and success of aerobic membrane bioreactors, and the pressures of increasing energy prices, more stringent regulations, and growing concerns regarding greenhouse gas emissions and environmental sustainability, research on AnMBRs for has increased substantially as reflected in Figure 2.3 from Dvořák et al. (2016). The first commercially successful AnMBRs came online in the early 2000s with the development of several demonstration-scale AnMBRs by Kubota in Japan treating night-soil, food processing wastewater, and distillery silage. This led to the construction of the first full-scale AnMBR by ADI in 2008 for the treatment of wastewater from salad dressing production (Bouman & Heffernan, 2016; Lin et al., 2013). To date, AnMBRs have been investigated for treatment of a wide range of wastes and wastewaters, including food processing, distillery, textile, slaughter house and other industrial wastewaters, as well as raw municipal wastewater, sewage sludge, animal manures, municipal solid waste, and landfill leachate (Lin et al., 2013). However, large-scale application of AnMBRs has been applied to a greater extent in industrial wastewater treatment with only a few large-scale applications in the context of municipal wastewater treatment (Chang, 2014; Dvořák et al., 2016; Lin et al., 2013).

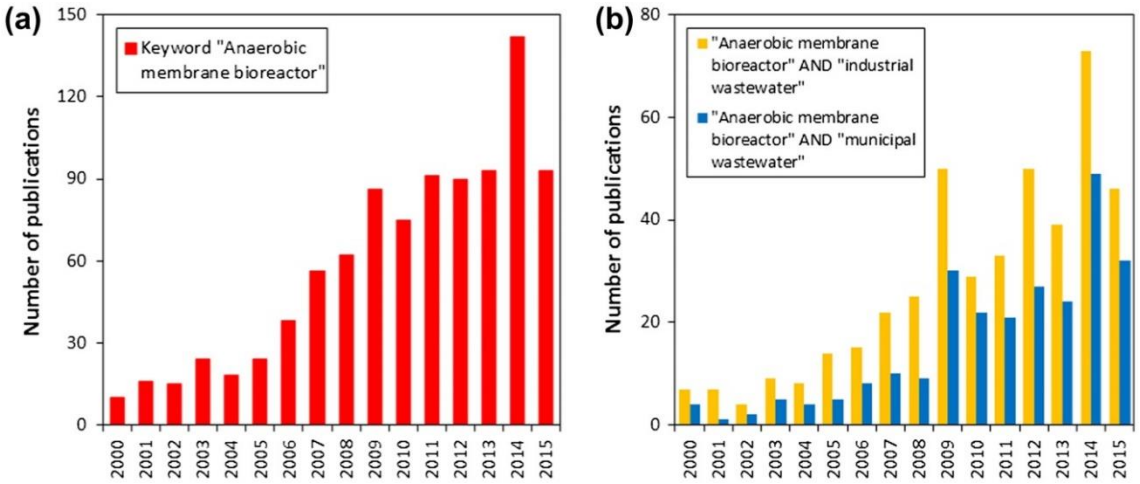


Figure 2.3. Number of publications from 2000 to September of 2015 identified in Scopus based on the key words (a) "AnMBR" only and (b) "AnMBR" in combination with "industrial wastewater" and "municipal wastewater" (From Dvořák et al., 2016).

Anaerobic treatment processes are most commonly carried out under mesophilic temperatures (37°C) (Stuckey, 2012). This makes AnMBRs an advantageous option for treating high organic strength, high solids content wastewater where the potential for energy recovery is more than the required energy for operation, and where retention of particulate solids by the membrane can offer improved solids degradation, methane yield, and effluent quality relative to other anaerobic treatment options. For low organic strength, high solids content wastewaters such as raw municipal wastewater (390-1230 mg COD/L) (Metcalf and Eddy), AnMBRs can be highly effective in terms of improving effluent quality and solids degradation, however the energy input for heating to mesophilic temperature is more than the energy that can be recovered as methane. In general, heating to mesophilic conditions is only possible for wastewaters with organic strengths above 4-5 g COD/L (Stuckey, 2012). Therefore, in order to achieve a positive energy balance, treatment of low strength wastewaters must be carried out under ambient temperature (Liao et al., 2006, Smith et al., 2015). Ambient temperature treatment, however, can potentially reduce process stability and decreased methane yields since microbial activity and hydrolysis rates decrease with temperature (Stuckey, 2012). In addition, the release of dissolved methane, a potent GHG, from AnMBR effluent to the atmosphere is a concern, particularly for low temperature operation since the solubility of methane increases with decreasing temperature (Smith et al., 2015). Decreased hydrolysis is particularly detrimental for municipal wastewater treatment since solids typically exert 30-70% of the total COD (Teo & Wong, 2014). Build-up of undigested solids can lead to increased cake formation and membrane fouling as well as decreased methane yield (Dvořák et al., Liao et al., 2006; Teo &Wong, 2014). Prashanth et al., (2006) investigated anaerobic treatment of synthetic wastewater containing various proportions of particulate and soluble organics and found that methane production decrease with increasing concentration of particulate solids. In general, membrane fouling is a significant challenge for AnMBRs, since anaerobic processes are meant to operate at higher organic loading rates and biomass concentrations compared to aerobic process, but higher reactor solids concentrations generally lead to increased membrane fouling (Visvanathan & Abeynayaka, 2012). Therefore, development of methods for reducing solids accumulation and mitigating membrane fouling are important areas of research for AnMBRs.

AnMBR Configuration

There are two basic AnMBR configurations: pressure driven cross-flow with an external membrane, and vacuum driven submerged membrane where the membrane may be placed either directly inside the reactor or outside in a separate membrane chamber in a side-stream configuration (Dvořák et al., 2016; Stuckey, 2012). The different AnMBR configurations are illustrated in Figure 2.4. Both configurations offer advantages and disadvantages in terms of energy consumption, membrane fouling and maintenance, and effects on the microbial community. In general, external cross-flow configurations consume more energy in order to achieve the high cross-flow velocities (2-4 m/s) needed for membrane filtration (Dvořák et al., 2016; Lin et al., 2016; Stuckey 2012). Although, the shear forces resulting from cross-flow filtration can be beneficial in terms of membrane fouling by eliminating cake formation on the membranes surface. At the same time, however, high shear forces put stress on the microbial community reducing methanogenic activity, and can disrupt larger sludge agglomerates and flocs, leading to a decrease in the overall particle size in the reactor and increasing the release of soluble microbial products (Padmasiri et al., 2007; Stuckey et al., 2012). While reducing average particle size in the reactor can initially lead to increased flux, it can also lead to increased membrane clogging and more frequent need for chemical cleaning (Dvořák et al., 2016; Lin et al., 2013).

Submerged membrane configurations, on the other hand, are associated with lower energy consumption for filtration, although additional energy must be applied for reducing cake formation on the membrane surface. (Chang, 2014; Dvořák et al., 2016; Lin et al., 2013, Martin et al., 2011). This is most commonly done via gas sparing with recirculated biogas, although alternative, less energy intensive methods are being investigated, such as application of adsorbents or other solid media into the reactor that can physically scour the membrane (Lin et al., 2013; Stuckey, 2012). In general, cake removal is less efficient in submerged configurations compared to cross-flow configuration and thus submerged membranes may become fouled more quickly leading to more frequent cleaning which can increase the rate of membrane deterioration and lead to higher costs. On top of that, submerged membranes are not as easily accessible and must be removed from the reactor prior to cleaning (Dvořák et al., 2016). Nevertheless, although cross-flow configurations have been the more popular configuration in past AnMBR studies, recent studies are showing greater interest in submerged membrane configurations due to their

potential for reducing the energy requirements associated with AnMBR processes (Stuckey, 2012).

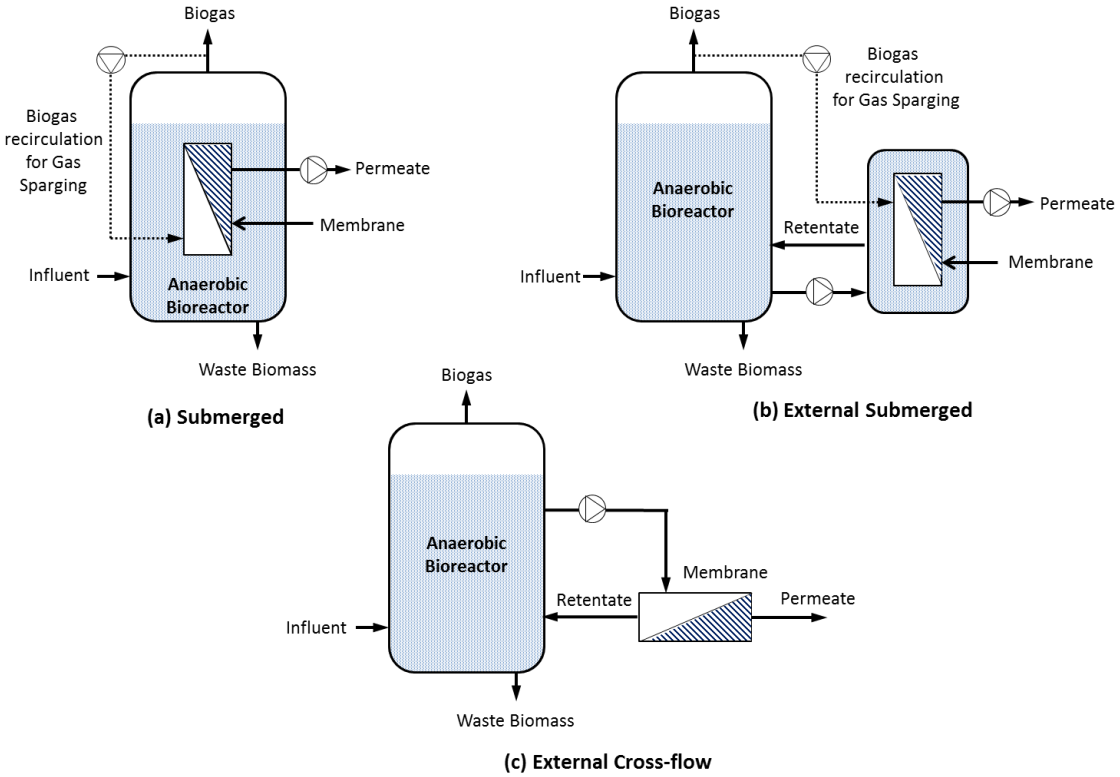


Figure 2.4. AnMBR configurations.

In either configuration the membrane may be made of polymer, ceramic, or metal. Polymer membranes are typically made of polyvinylidene (PVDF), polyethersulfone (PES), polyethylene (PE), polypropylene (PP), or polysulfone (PSF) (Dvořák et al., 2016, Lin et al., 2013; Stuckey, 2012). Currently, polymer membranes are the most commonly used due to their relatively low cost, while more expensive ceramic and metallic membranes, which can offer better permeability and stability toward chemical cleaning, are used for specialized applications (Dvořák et al., 2016, Lin et al., 2013). In addition, membranes can be either tubular, flat sheet, or hollow-fiber. Currently, hollow-fiber membranes are the most commonly used due to their high packing density and cost efficiency (Dvořák et al., 2016). Researchers have also investigated alternative, low-cost membrane materials, such as meshes and filter cloths, which could potentially reduce both capital and operating cost associated with AnMBRs (Meng et al., 2009), although further testing and large-scale demonstration is needed (Dvořák et al., 2016; Lin et al., 2013).

AnMBRs for Sewage Sludge Treatment

At wastewater treatment plants, sewage sludge represents a high strength, high solids content waste that may be particularly suited for treatment via AnMBR (Liao et al., 2006; Xu et al., 2010). Sewage sludge, which includes primary sludge (the solids fraction of primary clarified wastewater) and waste activated sludge, is a complex heterogeneous mixture of microorganisms, undigested organic material, inorganic material, and moisture (Tyagi & Lo, 2013). Typical solids contents for primary and activated sludge are 5-9% and 0.8-1.2%, respectively (Metcalf and Eddy). Around 50 g (dry wt.) of sludge are produced per capita per day representing a significant cost for wastewater treatment plants (Rulkens, 2008). It is estimated that sewage sludge treatment can account for 30-50% of total wastewater treatment cost (Rulkens, 2008; WERF, 2008). Many wastewater treatment plants currently treat sewage sludge via conventional anaerobic treatment. However, treatment via AnMBR, could provide increased methane recovery and reduced waste sludge production, potentially reducing the costs associated with sewage sludge treatment. Despite these benefits, only a handful of studies have looked at AnMBR treatment of sewage sludge (Aya & Namiki, 1992; Ghyoot & Verstraete, 1997; Kim & Somiya, 2001; Meabe et al., 2013; Murata et al., 1994; Pierkiel & Lanting, 2005; Pillay et al., 1994; Xu et al., 2010). Table 2.2 summarizes performance parameters for studies examining AnMBR treatment of sewage sludge as well as other similar high strength and high solids content waste streams.

Overall, AnMBRs treating sewage sludge have been able to achieve similar or higher solids removal (50-80%), at similar or higher OLRs (1-6.4 kg COD/m^3 d^{-1}) compared to conventional anaerobic treatment, while achieving very low effluent COD levels (less than 2.4 g/L). The greatest challenge for AnMBR treatment of sewage sludge, and other high solids content waste streams, is membrane fouling. The main mechanism for membrane fouling in these cases is cake formation due to the high concentrations of suspended solids that accumulate in the reactor. Again, this highlights the need for improving hydrolysis rates of high solids content wastes in order to reduce cake formation and mitigate membrane fouling (Liao et al., 2006).

Cross-flow membrane configurations have been applied in order to reduce membrane fouling, but in several cases have resulted in no or negative impact on process performance. For

example, Ghyoot and Verstraete (1994) found that although cross-flow configuration was able to mitigate cake formation and achieve a high quality permeate, shear stresses upset the microbial community and therefore no improvement in terms of OLR was made. Padmasiri et al. (2007) also had issues with shear stress finding that increased hydrolysis due to shear stress led to an increase in soluble fermentation products and VFA accumulation which limited the OLR that could be applied to the reactor. More recently, Meabe et al. (2013), in a pilot scale AnMBR treating sewage sludge at both mesophilic and thermophilic temperature, was able to achieve much higher OLR, 6.4 and 4.6 g COD/L d^{-1}, respectively and higher solids removal 72%, compared to conventional anaerobic treatment. In this case, thermophilic operation resulted in greater solids solubilization and thus better filtration, compared to mesophilic operation, but as a result, higher levels of VFA accumulation were observed, particularly propionate, and effluent COD levels were higher. Together, these studies highlight that while increasing hydrolysis via cross-flow filtration, thermophilic operation, or other means, is important in order to minimize cake formation and mitigate membrane fouling, strategies for preventing accumulation of soluble organics and VFAs are also needed in order to maintain process stability and high effluent quality.

Table 2.2. Summary of studies on AnMBR treatment of high strength and high solids content waste streams.

Type of Waste	Reactor Type	Scale[a]	Reactor Volume (m^3)	Temp. (°C)	HRT (d)	SRT (d)	OLR (kg COD/m^3 d^{-1})	MLSS (g/L)	Feed COD (g/L)	Feed TS (g/L)	Effluent COD (g/L)	COD Removal (%)	CH_4 Yield (L/g COD_{fed})	Ref.
Primary sludge	Upflow mixed	P	0.12	35	20	--	1.06	22-35	40.2	44.4	0.69	54	0.08-0.16	50
Primary sludge	CSTR	P	0.5	35	4.2-8.4	42-335	0.93[b]	30-55	--	10	0.01-0.03[b]	51-79[b]	0.27-0.29[b]	85
Primary sludge	CSTR	P	0.5	55	4.1-7.8	30-197	1.16[b]	30-55	--	10	0.1-1.8[b]	67-78[b]	0.24-0.31[b]	85
Waste activated sludge	USAB w/ membrane	L	0.008	37	6	80	--	10-33	--	--	--	52-60[b]	--	138
Sewage sludge	CSTR	P	0.025	35	7	50	4.6	69.6	31.6	29.1	0.27	72	0.242	76
Sewage sludge	CSTR	P	0.025	55	7	50	6.4	71	33.6	30.1	2.37	72	0.245	76
Sewage sludge	CSTR	P	0.55	35	1.7-11.8	4.2-70.5	--	18	--	6	--	59[b]	--	97
Sewage sludge	CSTR	L	0.004	25-50	6.7-20	--	0.17-1.35[b]	20-40	--	--	< 0.3	--	0.5[b]	11
Screened sludge	Semi-continuous CSTR	P	1.8	--	14	26	--	55	--	--	--	--	--	98
Coagulated raw sludge	VFA fermenter CSTR	P	0.076	35	0.5	10	4.6[c]	34	2.3[c]	6.8	1.3[c]	42[c]	--	64
MSW	2-phase CSTR/CSTR	L	0.010/ 0.003	35	2-15/1.6-2.3	--	0.5-16[b]	< 28.7	--/4-26	100/40	4-26/0.4-0.6	13-65/90	0.296[b]	119
MSW	2-phase CSTR/AF	L	--	--	1.5/--	20/--	3.75[b]	3.4-12.3/1-4.5	--	--	--	59-72/--	0.28	132
Swine manure	CSTR	L	0.006	37	6	--	1-3	20-40	--	--	2-2.5	96	--	90
Swine manure	CSTR	P	0.1	35	6	--	5	--	30	20	3	90	--	89
Swine manure	CSTR	F	200	35	10	--	3	--	30	20	2.4	92	--	89
Swine manure	2-phase CSTR/USAB	P	3/3	20/35	1-2/1-2	--	2.8-5.5/--	--	5.5	0.6	1.1	80	0.32	68
Chicken slaughterhouse	CSTR	L	0.007	30	1.2	--	4.3	22	5.2	2.4-4.7	< 0.5	90	0.12-0.32	46

[a]L = laboratory, P = pilot, F = full-scale

[b]Values are based on VSS instead of COD, [c]Values are based on TOC instead of COD

Bioaugmentation in Anaerobic Treatment Process

One strategy for targeting and improving the rate-limiting step of hydrolysis is through bioaugmentation with hydrolytic microorganisms. Bioaugmentation is the addition of specific microorganisms to a system in order to correct or enhance a desired process or activity (Ritmann & Whiteman, 1994; Herrero & Stuckey, 2015). Bioaugmentation has been used for a variety of reasons in several applications including soil and groundwater bioremediation, wastewater treatment, and anaerobic digestion of agricultural, industrial, and municipal solid wastes. In wastewater treatment, bioaugmentation has been applied most frequently in aerobic systems (Schauer Gimenez et al., 2010). In these cases it has been used to improve flocculation and degradation of specific substrates (Van Limbergen et al., 1998), as well as to increase the population of nitrifying bacteria after systems upsets resulting from pH or temperature fluctuations, uncontrolled biomass loss, or toxic events (Ritmann & Whiteman, 1994; Abeysinghe et al., 2002; Satoh et al., 2003; Head & Oleszkiewicz, 2005). In anaerobic treatment, bioaugmentation has been investigated for its benefits in overcoming shock loading or toxic events, improving reactor start-up, odor reduction, and degradation of specific compounds or substrates. Examples of previous studies that have investigated bioaugmentation in anaerobic treatment processes are summarized in Table 2.3.

Regarding hydrolysis, several studies have shown bioaugmentation with hydrolytic microorganisms to significantly improve solids reduction and methane production from a variety of substrates. Cirne et al. (2007) investigated the effects of bioaugmentation with an anaerobic lipolytic bacterium on anaerobic treatment of lipid-rich restaurant waste and were able to achieve 80% of methane yield in 30% less time compared to a non-bioaugmented control. Similarly, Cavaleiro et al. (2010) investigated the potential for improving long-chain fatty acid conversion to methane via bioaugmentation with *Syntrophomonas zehnderi* and were able to achieve a 26% increase in methane production rate with bioaugmentation. The majority of other studies investigating bioaugmentation for improved hydrolysis have focused on hydrolysis of cellulosic and hemicellulosic substrates. For example, Weiss et al. (2010) investigated bioaugmentation with mesophilic hemicellulolytic bacteria immobilized on activated zeolite as a method for enhancing biogas production from hemicellulose-rich substrates. Batch testing resulted in a 53% increase in methane production compared to a non-bioaugmented control. Costa et al. (2012) investigated the benefits of bioaugmentation with three different cellulolytic bacterial strains on

the hydrolysis and methane production from poultry litter. Of the three strains investigated bioaugmentation with *C. cellulolyticum* showed a significant positive effect on biogas production resulting in a 15% increase in cumulative methane production compared to a non-bioaugmented control. VFA concentrations also increased, lending to the conclusion that bioaugmentation with *C. cellulolyticum* enhanced hydrolysis and subsequent acidogenesis of the substrate. Batch tests indicated a solids concentration of 1% TS provided the best scenario in terms of methane production with higher solids concentrations resulting in inhibitory effects most likely related to VFA, alcohol and/or COD accumulation. Bioaugmentation with the other two cultures, *C. thermocellum,* and *C. saccharlyticus,* did not result in a significant increase in methane production, but did cause a significant increase in substrate solubilization. The authors concluded that in these cases methanogenesis was the rate-limiting step and attributed it to the fact that temperature was maintained at thermophilic conditions (55 and 65˚C) which was optimal for the growth of the bioaugmented species, but may have negatively influenced the mesophilic methanogenic inoculum. From this study, the authors believed that separation of hydrolysis from subsequent phases may be necessary for maximizing process efficiency.

Angelidaki et al. (2000) investigated both bioaugmentation as well as the addition of cellulase enzymes as separate methods for improving the methane potential of cattle manure. The authors found that treatment with hemicellulolytic and cellulolytic enzymes did not result in any significant increase in methane production compared to control conditions. In contrast, bioaugmentation with hemicellulose degrading bacterium B4 resulted in a 30% increase in methane production compared to non-bioaugmented controls. Romano et al. (2009), also found that the addition of cellulase enzymes had no significant improvement on methane yield or solids reduction for anaerobic digestion of Jose Tall Wheat Grass. In contrast, Roman et al. (2006), found that addition of cellulose and pronase E in combination increased particulate solids reduction and overall COD removal for anaerobic treatment of primary sludge. Finally, Teo et al. 2014, investigated hydrolytic enzyme addition in an AnMBR treating synthetic sewage finding that improvement in solids reduction (up to 22%) and methane production (up to 26%) were dosage dependent. The authors also noted that low enzymatic activities were detected throughout the study, likely due to the instability of free enzymes in the bioreactor environment.

These last studies highlight the potential of bioaugmentation as an effective alternative to enzyme augmentation as a means for improving hydrolysis. While many studies have

demonstrated benefits from the addition of hydrolytic enzymes in terms of increased methane production and solids reduction (Wawrzynczyk, 2003; Davidsson et al., 2007; Roman et al., 2006; Parmar et al., 2001), there are several drawbacks associated with enzyme application that make bioaugmentation a more attractive option. One of the major drawbacks is the high cost associated with commercial enzyme production. Other concerns are uneven distribution of enzymes or loss of enzyme activity due to entrapment within the solid waste matrix, thermal denaturation, active site inactivation, loss of cofactors or prosthetic groups, and inhibition (Ahuja et al., 2004; Aitken, 1993; Gianfreda & Rao, 2004). Also, in contrast to microorganisms, enzymes are not able to adapt to environments outside of their optimal range, and because they are soluble and unstable they can only be used once in solutions (Parawira, 2012). Parawira stated that, "Bioaugmentation offers the possibility of enzyme production over a longer period of time provided that the microorganism added is able to compete with the other microbes present in the reactor".

In that light, there are several factors that can influence the survival and productivity of bioaugmented microorganisms within the reactor, including substrate variability, environmental conditions, predation and/or competition among indigenous microorganisms, and wash-out (El Fantroussi et al., 2005; Herrero & Stuckey, 2015). In general, maintenance of bioaugmented microorganisms in the system is a challenge, and it is still a question whether or not adding limited quantities of externally cultured microorganism can improve long-term performance (Venkiteshwaran et al., 2016; Herrero & Stuckey, 2014). Although several studies have shown improvements in process performance as a result of bioaugmentation, few studies have investigated its application under continuous, long-term conditions, and of those that have, the benefits of bioaugmentation were often not sustained over time. Examples of this include studies by Nielsen et al. (2007) and Mladenovska et al. (2001), in which both authors were able to achieve significant increases in methane production from biofibers as a result of bioaugmentation with hydrolytic microorganisms, however, this increase diminished over time. In both cases, the suspected cause for the decline in methane production was wash-out due to an inability of the bioaugmented microorganisms to adapt and compete within the indigenous microbial community. As a method for sustaining the benefits of bioaugmentation, Martin-Ryals et al. (2015) investigated routine bioaugmentation with a proprietary cellulolytic bioculture in a two-phase mesophilic process treating sweet corn residuals. In this case, bioaugmentation increased

methane production by 56% in a bench-scale semi-continuous process, and in a sequencing batch test, routine bioaugmentation was found to significantly improve substrate solubilization (+25%) and methane production (+15%) compare to one-time bioaugmentation. Thus, regular repeated additions of the bioaugmented microorganism may be needed in order to maintain optimal process efficiency. Also, with respect to potential wash-out, bioaugmentation and AnMBR technology may complement each other, as the membrane can provide a means for retaining bioaugmented microorganisms in the system (Herrero & Stuckey, 2015). To the author's knowledge, no previous studies have investigated long-term application of bioaugmentation in an AnMBR making it a novel area of research.

Table 2.3. Examples of previous studies investigating bioaugmentation in anaerobic treatment processes.

Purpose for Bioaugmentation	Substrate	Bioaugmented Microorganism	Reactor Configuration	Results	Reference
Hydrolysis of lipids	Lipid-rich restaurant waste	Lipolytic bacterium: *Clostridium lundense*	Single-phase & two-phase batch test, mesophilic	Increased CH_4 production rates	Cirne et al., 2006
Hydrolysis of LCFA	Oleate	*Syntrophomonas zehnderi*	Batch test, mesophilic	26% CH_4 increase	Cavaleiro et al., 2010
Hydrolysis of xylose	Xylose powder	Hemicellulolytic bacteria	Batch test, mesophilic	53% CH_4 increase	Weiss et al., 2010
Hydrolysis of poultry litter	Poultry litter	Cellulolytic bacteria: *C. cellulolyticum*, *C. thermocellum*, *C. saccharlyticus*	Batch tests, mesophilic and thermophilic	Up to 74% increase in substrate solubilization, 15% increase in CH_4	Costa et al., 2012
Hydrolysis of wheat straw and cellobiose	Wheat straw and cellobiose	*Clostridium cellulolyticum*	Batch test using automated methane potential test system (AMTSII)	8-13% CH_4 increase	Peng et al., 2014
Hydrolysis cellulose and hemicellulose	Brewery spent grain	*Pseudobutyrivibrio xylanivorans* Mz5T, *Fibrobacter succinogenes* S85, *Clostridium cellulovorans*, *Ruminococcus flavefaciens* 007C	Batch test, mesophilic	Up to 17.8% CH_4 increase	Carter et al., 2015
Hydrolysis of cellulosic waste	Sweet corn processing residues	Routine bioaugmentation with a proprietary cellulolytic bioculture mixture	Bench-scale continuous, two-phase mesophilic, and sequencing batch	56% more CH_4, 25% more solubilization & 15% more CH_4 compared to one-time bioaugmentation	Martin-Ryals et al., 2015
Hydrolysis of biofibers	Cattle manure	Cellulolytic bacteria: *Caldicellusiruptor* & *Dictyoglomus*	Two-stage (68˚C/55˚C) batch test and bench-scale continuous	Increased CH_4 yields	Nielsen et al., 2007
Hydrolysis of biofibers	Cattle manure fibers	Hemicellulose degrading bacterium B4	Batch test, thermophilic (70˚C)	30% increase in CH_4 production	Angelidaki et al., 2000
Hydrolysis of biofibers	Cattle manure	Xylanolytic & cellulolytic bacteria	Bench-scale continuous, mesophilic	Increased CH_4 production rates	Mladenovska et al., 2001

Table 2.3 (continued)

Purpose for Bioaugmentation	Substrate	Bioaugmented Microorganism	Reactor Configuration	Results	Reference
Increase methanogenic activity	Synthetic wastewater	Methanogenic aerotolerant propionate enrichment culture	Single-phase semi-continuous in serum bottle, mesophilic	11% CH_4 increase	Venkiteshwaran et al., 2016
Increase methanogenic activity	Fodder beet silage	Compost: hydrogenotrophic methanogens	One-stage bench-scale continuous, mesophilic	2-4 fold shorter HRTs and 6% increase in biogas production	Neumann et al., 2011
Increase methanogenic activity	Pig and cattle manure	Methanoculleus bourgens	Single-phase CSTR, bench-scale	31% CH_4 increase	Fotidis et al., 2014
Improve low-temperature digestion	Xylose	*Clostridium sp.* PXYL1, *Methanosarcina sp* PWET1	Batch test, mesophilic	70-140% CH_4 increase	Akila, et al.2010
Overcome transient organic loading stress	Synthetic wastewater	Methanogenic aerotolerant propionate enrichment culture	Single-phase semi-continuous in serum bottle, mesophilic	50-150% CH_4 increase and shorter recovery time	Tale, et al. 2015
Improve recovery from toxic exposure to O_2	Synthetic municipal wastewater solids	H2-utilizing culture	Single-phase semi-continuous in serum bottle, mesophilic	25-60% increase in CH_4 production	Schauer- Gimenez et al., 2010
Improve reactor start-up	Pharmaceutical effluent	Anaerobic sludge collected from plant treating antibiotic effluent	Fluidized-bed reactor	Decrease in reactor start-up time and increase in COD removal	Sarvanane et al., 2001
Odor control	Anaerobic biosolids	Commercial product containing selected strains of *Bacillus*, *Pseudomonas*, & *Actinomycetes*	One-stage bench-scale continuous, mesophilic	29% increase in CH4 production, reduced generation of organic sulfide compounds	Duran & Tepe, 2006
Increase H_2 Formation	Corn straw	*Acetobacteroide hydrogenigenes*	Batch test, mesophilic	19-23% CH_4 increase	Zhang et al., 2015
Pentachlorophenol Degradation	Wastewater	*Desulfitobacterium frappieri* PCP-1	USAB	53% increase in removal rate	Guiot et al., 2002

Application of Sorption Media in Anaerobic Treatment Process

Application of adsorbent media or ion-exchange resins in AnMBR treatment processes has the potential to improve process performance in a number of ways including mitigating effects associated with organic loading fluctuation and VFA accumulation, improving effluent quality, and reducing membrane fouling. In terms of organic loading, VFA accumulation, and effluent quality, the adsorbent or ion-exchange resin can act as a temporary physio-chemical sink for soluble organic material reducing their concentration in the bulk liquid via sorption onto the adsorbent or ion-exchange media. Sorption processes require little or no energy inputs and generally only require seconds or minutes of contact time with the solution to effectively reduce bulk liquid absorbate concentrations, which is significantly faster than biological processes (Metcalf & Eddy, 2003). As a result, application of adsorbent or ion-exchange media during times of organic overload or VFA accumulation can reduce the stress on the microbial community, giving it time to adjust to the new environmental conditions and/or degrade the excess organic material, while at the same time, reduce the amount of soluble organics that leave the AnMBR in the effluent. In terms of membrane fouling, the addition of adsorbents or ion-exchange resin can benefit in two ways: by disrupting the cake layer via physical scouring of the membrane surface, and by sorption of potential foulants, such as soluble organics, colloids, and soluble microbial products, that could otherwise clog membrane pores and contribute to membrane fouling (Stuckey, 2012). In addition, application of natural zeolites, such as clinoptilolite, have also been widely investigated in wastewater treatment applications a means from removing ammonium ion in order to reduce toxicity or recover nitrogen.

Activated Carbon and Ion-Exchange Resins

Activated carbon has been the most commonly used adsorbent material in wastewater treatment applications (Metcalf & Eddy, 2003). Activated carbon is essentially chard organic material that has been "activated" via exposure to oxidizing gases such as steam and CO_2 at high temperature (Metcalf & Eddy, 2003). These gases create pores within the char particle which increase the surface area of the particle. The resulting surface properties are dependent on the initial material used, and the preparation process. Activated carbon is available in either a granular (0.1- 2.36 mm diameter) or powered form (5-50 µm diameter), with average surface areas of 700-1300 and 800-1800 m^2/g, respectively (Metcalf & Eddy, 2003). Adsorption occurs

in four steps: (1) bulk solution transport, (2) film diffusion transport, (3) pore transport, and (4) adsorption. Adsorption can occur on the surface of the adsorbent or within its pores as shown in Figure 2.5. Adsorption forces included: coulombic-unlike charges, point charge and a dipole, dipole-dipole interactions, point charge and neutral species, London or van der Waals forces, covalent bonding with reaction, hydrogen bonding (Metcalf & Eddy, 2003).

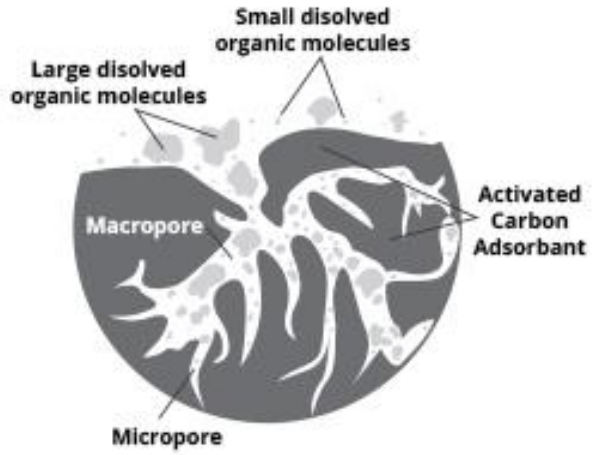


Figure 2.5. Adsorption of organic molecules onto activated carbon (From AquaCache).

Ion-exchange is another type of sorption process, in which ions of a given species are displaced from an insoluble exchange material by ions of a different species from the surrounding solution (Metcalf & Eddy, 2003; Wheaton & Lefevre, 2000). Naturally occurring ion-exchange materials, or zeolites, include complex aluminosilicates which are often used for water softening, and clinoptilolite which is often used for ammonium ion removal. Synthetic ion-exchange materials are typically resins that consists of a cross-linked polymer matrix with a relatively uniform distribution of ion-active sites throughout (Wheaton & Lefevre, 2000). A cation exchange resin will have a negatively charged matrix and exchange cations, while in contrast an anion exchange resin will have a positively charged matrix and exchange anions. Figure 2.6 provides a visual representation of the ion-exchange process for an anionic exchange resin. The majority of ion-exchange resins are spherical in form (beads) with a particle size distribution around 0.3 - 1.2 mm (Wheaton & Lefevre, 2000). There are five types of synthetic ion-exchange resins: (1) strong-acid cation, (2) weak-acid cation, (3) strong-base anion, (4) weak-base anion, and (5) heavy-metal selective chelating resins (Metcalf & Eddy, 2003). The properties of each resin type are described in Table 2.4.

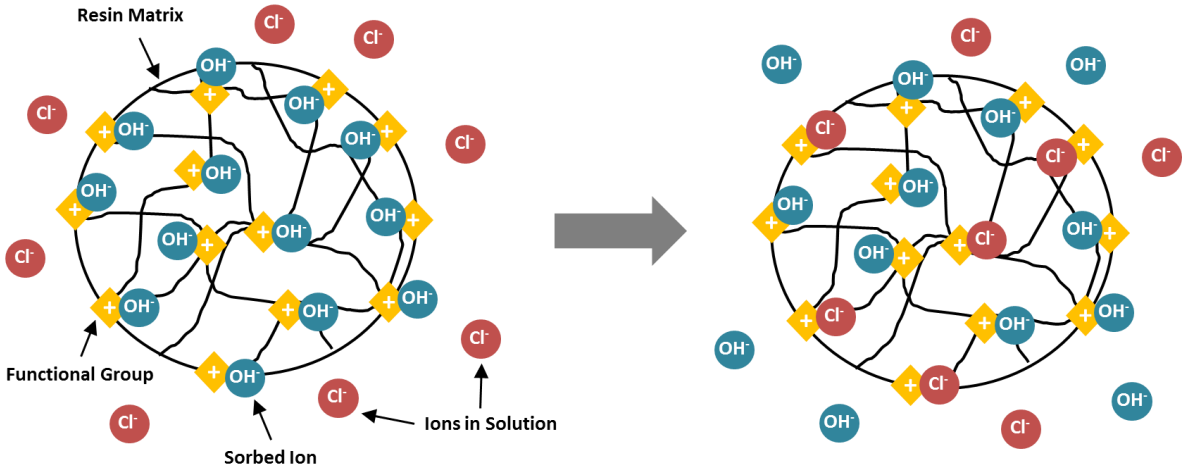


Figure 2.6. Visual representation of anion exchange process.

Table 2.4. Classification of ion-exchange resins (adapted from Metcalf & Eddy, 2003).

Type of Resin	Characteristics
Strong-acid cation	Behave like strong acids. Highly ionizable in both acid (R-SO_3H) and salt (R-SO_3Na) forms. Exchange capacity is independent of pH range.
Weak-acid cation	Behave like weak acid. Functional group is typically carboxylic group (-COOH). Weakly dissociate and degree of dissociation is strongly dependent on pH.
Strong-base anion	Highly ionizable in both acid and salt forms. Have strong base functional group (e.g. OH-, Cl-). Exchange capacity is independent of pH range. Classified as Type I or Type II depending on functional group.
Weak-base anion	Weak-base functional group. Sorb strong acids and do not split salts. Degree of dissociation is highly dependent on pH with minimum exchange capacity above pH 7.0
Heavy-metal selective chelating resins	Behave like weak-acid cation exchange resins with a high degree of selectivity for heavy-metal cations. Functional group is usually EDTA.

The chemistry of ion-exchange, using an anion exchange as the example, can be represented by the following equilibrium equation:

$$nR^+A^- + B^{-n} \leftrightarrow R_n^+B^{-n} + nA^-$$

where R^- is the functional cationic group attached to the ion-exchange resin and A and B are anions in solution.

The selectively, or affinity of an ion-exchange resin for a particular ion depends on the properties of that ion and its concentration in solution. An example of the typical order of affinity for various cations and anions, on strong-acid and strong-base ion-exchange resin respectively, is as follows (Metcalf & Eddy, 2003):

$$Li^+ < H^+ < Na^+ < NH_4^+ < K^+ < Rb^+ < Ag^+ < Mg^{2+} < Zn^{2+} < Co^{2+} < Cu^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+}$$

$$HPO_4^{2-} < CO_3^{2-} < OH^- \text{ (Type I)} < SO_4^{2-} < CH_3COO^- < HCO_3^- < OH^- \text{ (Type II)} < Cl^- < Br^- < NO_3^-$$

Application of Activated Carbon and Ion-Exchange Resins in AnMBRs

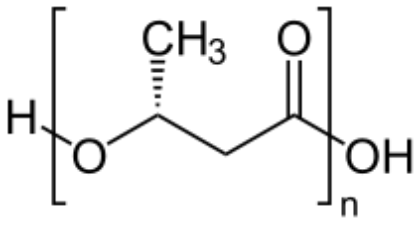
Previous literature has shown that the addition of activated carbon can be beneficial in improving COD removal, mitigating organic shock-loading events, and improving membrane flux (Akram & Stuckey, 2008; Hu & Stuckey, 2007; Park et al., 1999; Trzcinski, 2009; Yoo et al., 2012). One of the first studies of adsorbent addition was conducted by Park et al. (1999) who found that addition of up to 5 g/L of powdered activated carbon (PAC) enhanced both membrane flux and COD removal. Since then, addition of powered and granular activated carbon (GAC) in AnMBRs treating wastewater has been investigated by several researchers. Akram and Stuckey (2008) found that the addition of PAC to a submerged AnMBR treating synthetic wastewater improved start-up and performance during shock-loading by buffering VFAs. In this case, PAC was observed to have adsorbed slowly biodegradable low and high molecular weight (MW) residual COD as well as fine colloids, thereby improving overall COD removal and flux. Batch tests from this study also indicated that PAC addition could improve methane production rate and yield. Hu and Stuckey (2007) investigated the effect of both GAC and PAC on the performance of an AnMBR treating synthetic wastewater. The authors found that addition of PAC significantly increase COD removal, whereas the addition of GAC had limited effect on COD removal. This was attributed to the larger surface area of PAC related to GAC, which allowed more colloids and macromolecules to attach to it, though VFAs were only limitedly adsorbed. Additionally, specific methane potential was higher in the condition with activated carbon addition, which was believed to be a result of the activated carbon acting as a growth support for the microorganisms, protecting them from high shear conditions. This study was also the first to address whether the added activated carbon would eventually saturate over long-term operation or whether it would be bioregenerated and continue to be effective over long periods of

time. The authors determined that because the observed benefits of increased COD removal and higher membrane flux persisted for over three months, bioregeneration of the PAC was in fact taking place, and adsorbed solutes were being degraded overtime regenerating the surface for new solutes to adsorb (Hu & Stuckey, 2007; Stuckey, 2012).

While several studies have shown the benefits of activated carbon addition to AnMBRs, fewer studies have investigated the addition of ion-exchange resins (Akram, 2007; da Silva & Miranda, 2013). Ion-exchange resins can provide the same benefit of adsorption of soluble organics, and in fact, anion-exchange resins may provide better sorption of VFAs than activate carbon. This is because activated carbon will adsorb mostly neutral compounds and a much smaller amount of positively and negatively charged organics compounds, whereas anion-exchange resins can selectively adsorb negatively charged organics compounds, i.e. VFAs. This was evident in the study by Hu and Stuckey (2007), where the addition of PAC was found to significantly increase overall COD removal, but only limited adsorption of VFAs was observed. Similarly, da Silva and Miranda (2013) achieved greater adsorption of VFAs from fermentation broth with a weak base ion-exchange resin than with activated carbon. Rebecchi et al. (2016) investigated four candidate ion-exchange resins for volatile fatty acid recovery from the effluent of anaerobic acidogenic digestion of grape pomace, and determined that the weak-base resin, Amberlyst A21 was the most effective candidate for application in adsorption/desorption processes for VFA recovery, with greater affinity for long chain VFAs relative to short chain VFAs. Finally, Akram (2007) used the weak cation exchange resin, Amberlite IRC-50, in a submerged AnMBR treating synthetic wastewater to buffer peaks in VFA concentration resulting from organic shock-loading (20 kg COD/L). The author found that addition of the resin mitigated pH fluctuation and stabilized reactor performance in spite of increased VFA concentrations during shock-loading. After a period of acclimation and with a sufficient quantity of the resin (5 g/L), VFA levels were reduced. Addition of the resin also reduced cake formation and improved membrane flux via scouring of the membrane. Other benefits of ion-exchange resins relative to activated carbon include faster desorption kinetics due to more adsorption sites near the surface, which facilitates release of organics when microbes have depleted the bulk water concentrations, and because ion-exchange resins are flexible polymer structures concerns about membrane damage due to abrasion are reduced.

Polyhydroxyalkanote Production from Organic Waste

Conversion of organic material to valuable biomaterials, rather than methane, is an alternative method for resource recovery from organic waste, which may offer significant economic benefits. Polyhydroxyalkanote (PHA) is one example of a biomaterial that can be produced from organic waste streams. Polyhydroxyalkanotes, shown in Figure 2.7, are biodegradable polyesters that are stored as granules in the bacterial cell cytoplasm under stressed conditions (Serafim et al., 2008). Biopolymers, such as PHA, can be used as an alternative to petroleum based polymers providing the advantage of conserving fossil fuel resources and reducing CO_2 emissions, making them an important innovation in terms of sustainable development (Bugnicourt, 2014). Due to their biodegradability, biocompatibility, chemical-diversity, and being manufactured from renewable carbon resources, PHAs are considered one of the most promising biopolymers as an alternative to petroleum based plastics.

(a) 

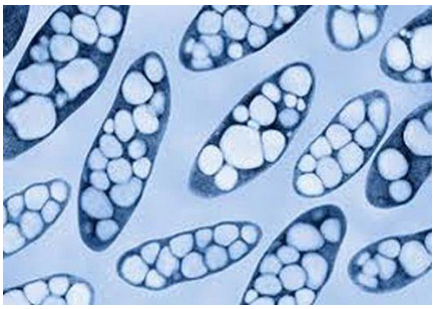
(b) 

Figure 2.7. Polyhydroxyalkanote (a) polymer, and (b) granules stored within the cell cytoplasm (Photo source: www.coolhunting.com/tech/switchgrassderi).

Presently, industrial processes for PHA production are based on the use of pure cultures of selected microbial strains that then requires use of single, pure substrates for cultivation (Setiadi et al., 2015; Villano et al., 2014). This makes PHA production expensive and uncompetitive with synthetic thermoplastics, due to the costs of culture maintenance, substrate formulation, and both substrate and reactor sterilization (Ivanov et al., 2015). The current PHA price, which depends on monomer composition and is usually higher for copolymers, ranges from $2.3-5.3/kg compared to $1/kg or less for conventional petroleum-based polymers (Valentino et al., 2017). Therefore, there is a need to reduce the cost of PHA production in order for it to be able to compete with conventional petroleum based plastics. This has motivated the investigation of mixed microbial cultures (MMC) coming from waste as a potential low cost

alternative to pure culture PHA production, as it does not require maintaining sterile conditions and can be combined with the use of low-cost waste feedstocks (Valentino et al,, 2017; Villano et al,, 2014).

PHA production from MMC is based on the natural selection of PHA accumulating microorganisms via application of selective pressures to the community, such as carbon or nutrient limitation. Typically the process is carried out in three steps as illustrated in Figure 2.8: (1) fermentation of the organic waste feedstock to produce VFAs, (2) selection of PHA accumulating bacteria on the VFA-rich substrate using a feast/famine feeding regime, and (3) accumulation of PHA within the selected biomass under nutrient limited conditions (Korkakaki et al,, 2016; Serafim et al,, 2008; Valentino et al,, 2017). Many types of wastewater can be used for the production of PHAs, so long as they contain high concentrations of fermentable COD, with relatively low nitrogen, solid concentrations, and toxicity (Tamis et al., 2014). From this perspective, food and paper industry effluents have been considered one of the most suitable substrates for waste-based PHA production. Other more complex waste streams such as leachate from composting, the organic fraction of municipal solid waste, and municipal wastewater could also be utilized for PHA production, but additional challenges must be overcome due to the relatively high nitrogen content and the presence of solids (Tamis et al., 2014). The process for PHA production from MMC and organic waste is discussed in more detail in the following section.

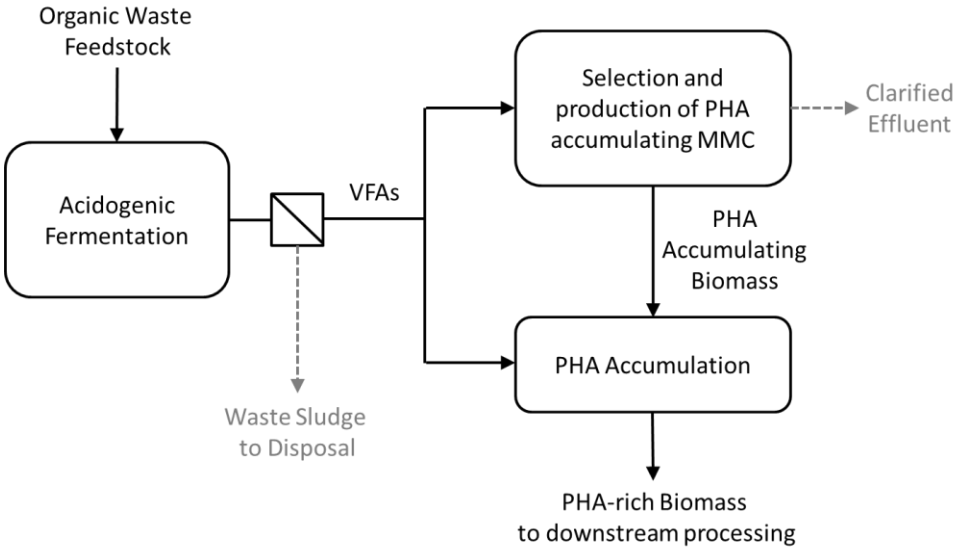


Figure 2.8. Most applied scheme for three phase MMC PHA production from organic waste (adapted from Valentino et al., 2017).

Mixed Microbial Culture PHA Production

Fermentation

VFAs have been shown to be the preferred substrate for MMC PHA production because they are both readily made available via anaerobic fermentation of organic material, and are efficiently converted into PHA (Luengo et al., 2003, Valentino et al., 2017). The first step in MMC PHA production is fermentation of the organic feedstock to produce a VFA-rich liquid effluent. This is carried out just as in anaerobic digestion, where particulate organic material is first hydrolyzed into soluble intermediates, via hydrolytic bacteria, and then further converted into VFAs and other acidogenesis intermediates via acidogenic, or fermentative bacteria. In this case, the process should be operated such to avoid methanogenesis, which would lead to loss of the desired VFAs to methane. Most fermentation processes are carried out in continuous stirred-tank reactors, therefore strategies to avoid methanogenesis include operating the reactor at a low SRT, low temperature, and/or low pH. Furthermore, operating parameters should be optimized to achieve maximum VFA production for a given feedstock. In addition, operating parameters can have an effect on the composition of the VFAs produced, which in turn can impact the quality of the resulting PHA. For example, Bengtsson et al. (2008) and Albuquerque et al. (2007) observed that fermentation HRT and pH had an effect on the resulting VFA composition. While acetate was in all cases the predominate VFA, both authors observed an increase in propionate concentration with increasing HRT and pH, while lower pH resulted in increased concentrations of butyrate and valerate. Prior to application in the following phases of PHA production, the resulting VFA-rich fermentation effluent may need to be filtered to remove particulate organic material which may interfere or inhibit the biomass selection and PHA accumulation phases.

Biomass Selection

The second phase of MMC PHA production is selection of the MMC biomass. Selection of a culture with a high PHA storage capacity is one the most important challenges in MMC PHA production processes (Serafim et al., 2008). This is typically achieved via an aerobic feast/famine feeding strategy using activated sludge as the starting biomass. The concept of aerobic feast/famine was first proposed by Majone et al. (2006) (Serafim et al., 2008). In this process, the microbial culture is exposed to a dynamic feeding regime of alternating periods of high and low substrate, i.e. VFA, concentrations. As shown in Figure 2.9, the biomass is initially

fed a given amount of substrate which begins the ‘feast’ period. After sometime, the bacteria will have consumed all or most of the substrate thus entering the ‘famine’ period. During this period, bacteria who are able to store organic carbon as PHA will have the advantage and be able to survive using their stored PHA for energy and growth, while non-PHA accumulating bacteria will die out (Lee, 1996; Van Loosdrecht et al., 1997). Thus, with multiple cycles of feast and famine, a greater abundance of PHA accumulating bacteria can be cultivated. Typically the feast period is limited to around 20% or less of the total cycle time (Valentino et al., 2017). At lab scale, this process is usually carried out in sequencing batch reactors, however, continuous flow configurations will likely be more applicable for full-scale processes (Valentino et al., 2017).

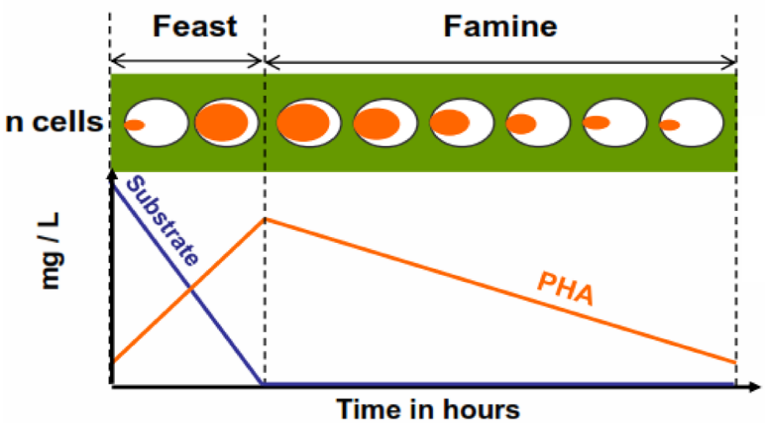


Figure 2.9. Feast/famine process for selection of PHA accumulating bacteria **(**Fatone, et al., 2014).

While aerobic dynamic feeding (ADF) has been the most commonly applied method for PHA selection, applying the feast/famine approach under anaerobic/aerobic or anoxic/aerobic conditions have also been investigated. Such strategies can reduce the amount of aeration required, which can in turn reduce energy requirements, and in the case of anoxic/aerobic, can potentially provide simultaneous nutrient removal. In general, high nutrient concentrations can be a problem for MMC PHA production. Anterrieu et al. (2014), applied anoxic feast/aerobic famine cycling to the treatment of a nutrient-rich sugar beet factory wastewater, and were able to achieve nitrification, denitrification and phosphorous removal along with selection of PHA-accumulating biomass. Similarly, Basset et al. (2016) applied an aerobic feast/anoxic famine process in the biomass selection phase achieving nitrification/denitrification, with the internally stored PHA driving denitrification during the famine period. However, the authors found that the presence of non-VFA soluble COD was believed to have negatively impacted the downstream

PHA accumulation phase. Other factors that can impact the biomass selection phase include operating parameters such as SRT, OLR, feast-to-famine ratio, cycle length, temperature, and substrate composition. To date, no comprehensive multi-parametric model has been developed for predicting the performance of the PHA selection phase or MMC PHA production in general.

PHA Accumulation

The last phase of the three-phase MMC PHA process is accumulation of PHA in the previously selected PHA-accumulating biomass. This typical involves exposing the biomass to conditions of extended feast. This can be done in batch mode, with a single addition of substrate, or with multiple additions of substrate. In this phase, limiting nutrient concentrations is especially important in order to minimize biomass growth, and focus substrate utilization to PHA storage. Optimal N/COD and P/COD ratios that have been reported for PHA-accumulation range from 2.0-15 mg/g and 0.5-3.0 mg/g respectively (Valentino et al., 2017).

PHA Production from Complex Organic Wastes

Several studies have shown the feasibility of using mixed microbial cultures and various waste feedstocks to achieve PHA enriched biomass. Table 2.5 provides a summary of operating conditions and PHA yield for several studies that investigated MMC PHA production using synthetic and real wastewaters. The highest PHA yield reported in Table 2.5, 89 g PHA/g VSS, was achieved using acetate as the feedstock under nutrient limited conditions in the study by Johnson et al. (2009). This was comparable to maximum PHA yields achieved in conventional PHA production processes using pure cultures, where up to 90% PHA per gram of cell dry weight has been achieved (Serafim et al., 2008). With industrial wastewaters such as paper mill, olive mill, molasses, and sugar factory wastewaters, researchers have been able to achieve 24-84% of PHA in the biomass. However, with more complex waste streams such as municipal wastewater and municipal solids waste, lower PHA yields have been achieved ranging from 9-53% of VSS. In general, the presence of non-VFA COD and high nutrient concentrations in these waste streams can be problematic for biomass selection and PHA accumulation. Morgan-Sagastume et al. (2010) investigated MMC PHA production from fermented municipal waste activated sludge (WAS) and were able to achieve 8-19% PHA in the biomass after accumulation. However, when the authors applied a synthetic feed mixture in the PHA accumulation phase, which contained the same composition of VFAs as the fermented WAS, but contained either

zero or the same concentration of nutrients, the authors observed PHA yields of 0.23-0.29 and 0.13-0.23 g PHA/g TSS, respectively. Thus, feeding the same concentration of VFAs, but without the complex matrix of fermented WAS, resulted in higher PHA accumulation, while the presence of nutrients was found to limit PHA accumulation. The authors concluded that the lower PHA storage rates were likely associated with active biomass-growth during the PHA accumulation phase induced by high levels of nutirents, and non-VFA organics, which made up 40-50% of soluble COD.

As means for addressing high nutrient content, precipitation of struvite has been investigated. Mengmeng et al. (2009) investigated PHA production from alkaline fermented sewage sludge. Prior to application of the fermented sludge substrate for PHA accumulation, magnesium chloride hexahydrate ($MgCl_2*6H_2O$) was added to the liquid to precipitate struvite ($MgNH_4PO_4*6H_2O$), thereby removing phosphorus and nitrogen from the solution. The authors were able to achieve PHA yields up to 56.5%, with 91.5% removal of soluble organic phosphate and 63% removal of ammonium. Addition of natural zeolites or other ion-exchange resins is another possibility for addressing the issue of high nutrient concentrations, which to the authors knowledge, has not been investigated in the context of PHA production from organic waste. Regeneration of the ion-exchange media could provide a nutrient-rich effluent with potential for further value-added byproduct production such as fertilizer or algal biomass production.

Finally, to date, few studies have investigated the use of OFMSW as a feedstock for MMC PHA production. Korkakaki et al. (2016) investigated the use of leachate from OFMSW as the substrate in both the biomass selection and PHA accumulation phases, achieving a PHA content of 29%. However, when the authors applied a synthetic VFA mixture as the substrate for biomass selection, they were able to achieve PHA yield up to 78%. The authors concluded that selecting the biomass in a clean VFA stream and using the complex waste stream for PHA accumulation could improve process efficiency. Basset et al. (2016) investigated PHA production from fermented OFMSW in a novel aerobic/anoxic biomass selection process, achieving 93% ammonia removal and 98% nitrite removal. However, the authors were only able to achieve up to 10.6% PHA in the biomass since high nutrient concentrations promoted bacteria growth in the accumulation phase. In short, achieving high PHA yields with complex waste streams is a significant challenge due to the issues of high solids and nutrients content (Serafim et al., 2008; Tamis et al., 2014).

Table 2.5. Examples of operating conditions and PHA yields for studies investigating MMC PHA production from synthetic feeds and organic wastes.

Feedstock	*Biomass Selection Phase*							*PHA Accumulation Phase*				
	Process Scheme	SRT (d)	OLR (g COD/ L d^{-1})	Cycle Length (h)	Temp. (ºC)	pH	COD:N:P	Feast Length (%)	Feeding Regime	COD:N:P	PHA Yield (g PHA/g VSS)	Storage Yield (COD/COD)	Ref.
Synthetic Feeds													
Acetate	Aerobic SBR	4	--	4	20-30	7.0	100:2-9:0.1-1.2	6	Batch, single feeding	100:0:0.1-1.2	0.69-0.89	--	60
Acetate	Aerobic SBR	8	--	5	20	--	100:2-12-2	--	Batch, single feeding	100:2-12:2	0.38-0.43	0.51-0.69	12
Acetate	Anaerobic/ aerobic SBR	10	--	8	18-20	7.0	100:2.5-5:1	--	Batch, single feeding	100:0-5:0.1-1.0	0.12-0.59	--	135
VFA mix	Aerobic SBR	1	8.5-40.8	0.42-2	25	7.5	100:5.5:1.5	17-64	Batch, single feeding	100:4.8:1	0.23-0.34	0.16-0.32	128
VFA mix	Aerobic SBR	1	8.5	2	25	7.5-9.5	100:5.5:1.5	19-22	Batch, single feeding	100:4.8:1	0.24-0.38	0.27-0.32	129
VFA mix	Aerobic SBR	1	8.5	3-8	25	7.5	100:4.5:1.0	19.7-28	Batch, single feeding	100:0.08:--	0.14-0.51	0.06-0.53	122
VFA mix & lactate	Aerobic SBR	1	8.5-31.25	2	25	7.5	100:4-7:0.7-1.4	9.5-52.5	Batch, single feeding	100:4.8:1	--	0.62	37
Glucose	Aerobic SBR	--	1.5-4.5	--	28	7.0	100:5:3	--	Batch, single feeding	100:2.2-6.6:1.1-3.3	0.38-0.54	--	102
Industrial Wastes													
Paper mill ww	Aerobic SBR	7	--	--	30	7.5	--	--	Batch, single feeding	100:0.03-4.7:0-1.9	0.43-0.48	0.33-0.67	15
Paper mill ww	Aerobic SBR	2	4.5	24	30	7.0	100:6:1.5	2-4	Batch, single feeding	100:0:1.2	0.77-0.84	0.80	61
Sugar cane molasses	Aerobic SBR	10	2.2-4.4	12	23-25	8.0	100:8:1	17-50	Batch, single feeding	100:0.03-0.5:--	0.33-0.61	0.66-0.84	6
Sugar cane molasses	Aerobic SBR	4	2	12	23-25	8.0	100:10:1	23	Batch, multiple feedings	--	0.52	0.62	39

Table 2.5 (continued)

Feedstock	*Biomass Selection Phase*							*PHA Accumulation Phase*				
	Process Scheme	SRT (d)	OLR (g COD/ L d^{-1})	Cycle Length (h)	Temp. (ºC)	pH	COD:N:P	Feast Length (%)	Feeding Regime	COD:N:P	PHA Content (g PHA/g VSS)	Storage Yield (COD/COD)	Ref.
Sugar cane molasses	Anaerobic/ aerobic SBR	10	47.4	8	30	7.7	100:3:1	--	Batch, multiple feedings	--	0.32-0.37	0.94-1.0	16
Molasses	Aerobic SBR	10	0.09	12	23-25	8-9	100:8:1	24	Batch, multiple feedings	100:6.7-7.5:--	0.57-0.58	0.57-0.71	145
Sugar factory ww	Anoxic/ aerobic SBR	16	0.3-0.5	8	25	6.5	100:11:--	--	Batch, single feeding	100:2:0.1	0.24	0.33	9
Olive mill ww	Aerobic SBR	1	2.4-8.4	6	25	7.5	100:3:1	10-24	Batch, multiple feedings	100:2:--	0.30	0.45	20
Olive mill ww	Aerobic SBR	1	8.5	2	25	7.5	100:5.5:1.5	--	Batch, single feeding	--	0.8	0.35	14
Municipal Wastes													
Primary sludge	Anaerobic/ aerobic SBR	5	--	24	18	8-9	--	--	Batch, single feeding	--	0.53	--	29
Municipal ww & activated sludge	Aerobic SBR	1-2	3	1.75	22.5	--	100:10-12:1.5	13	Batch, Feed-on-demand	100:10:4.5-6.0	0.27-0.38	0.25-0.38	81
Sewage sludge	Anoxic/ aerobic SBR	12-15	0.7-1.4	6	21-27	--	100:12-25:--	--	Batch, single feeding	100:9.7:2.1	0.19	--	45
Waste activated sludge	Aerobic SBR	3.1-6.4	6-12	4	35	--	100:9.5-11.5:1.5	< 20	Batch, Feed-on-demand	100:9.5-11.5:1.5	8-19	0.4	82
OFMSW & primary sludge	Aerobic/ anoxic SBR	25	0.26	3.3	--	--	100:5.5:1.2	10-15	Batch, Feed-on-demand	100:5.5:1.2	0.11	0.08	13
OFMSW	Aerobic/ anoxic SBR	25	0.2	3.3	--	--	100:4.5:0.42	10-15	Batch, Feed-on-demand	100:4.5:0.42	0.09	0.003	13
OFMSW leachate	Aerobic SBR	1	--	12	20	7.0	100:18:2.2	1-58	Batch, Feed-on-demand	--	0.29	0.3	65

References

1. Abeysinghe, D., D. De Silva, D. Stahl and B. Rittmann. (2002). The effectiveness of bioaugmentation in nitrifying systems stressed by a washout condition and cold temperature. *Water Environment Research* 74(2): 187-199.
2. Ahuja, S., G. Ferreira and A. Moreira. (2004). Utilization of enzymes for environmental applications. *Critical reviews in biotechnology* 24(2-3): 125-154.
3. Aitken, M. D. (1993). Waste Treatment Applications of Enzymes – Opportunities and Obstacles. *The chemical engineering journal* 52(2): B49-B58.
4. Akram, A. (2007). *Stability and performance improvement of a submerged anaerobic membrane bioreactor (SAMBR) for wastewater treatment*. PhD Dissertation. Imperial College London.
5. Akram, A., & Stuckey, D. C. (2008). Flux and performance improvement in a submerged anaerobic membrane bioreactor (SAMBR) using powdered activated carbon (PAC). *Process Biochemistry*, *43*(1), 93–102.
6. Albuquerque, M. G. E., Torres, C. A. V, & Reis, M. A. M. (2010). Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: Effect of the influent substrate concentration on culture selection. *Water Research*, *44*(11), 3419–3433.
7. Akila, G., and Chandra, T.S. (2009). Stimulation of biomethanation by Clostridium sp. PXYL1 in coculture with a Methanosarcina strain PMET1 at psychrophilic temperatures. *Applied Microbiology*.108:204-213
8. Angelidaki and B. Ahring. (2000). Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. *Water science and technology* 41(3): 189-194.
9. Anterrieu, S., Quadri, L., Geukink, B., Dinkla, I., Bengtsson, S. (2014). Integration of biopolymer productionwith process water treatment at a sugar factory. *New Biotechnology*. 31:308-323
10. AquaCache. (2017). AquaStream Modular Filter Arrays. AquaCache. http://aqua-cache.com/components/wpu
11. Aya, H., & Namiki, K. (1992). Anaerobic Digestion of Sewage Sludge by Membrane Separated Bioreactor. *Mizu Kankyo Gakkaishi (Japanese)*, (15), 187.
12. Basak, B., Ince, O., Artan, N., Yagci, N., Ince. B.K. (2011). Effect of nitrogen limitation on enrichment of activated sludge for PHA production. *Bioprocess Biosyst Eng*. 34:1007–1016
13. Basset, N., Katsou, E., Frison, N., Malamis, S., Dosta, J., & Fatone, F. (2016). Integrating the selection of PHA storing biomass and nitrogen removal via nitrite in the main wastewater treatment line. *Bioresource Technology*, *200*, 820–829
14. Beccari, M., Bertin, L., Dionisi, D., Fava, F., Lampis, S., Majone, M., Villano, M. (2009). Exploiting olive oil mill effluents as a renewable resource for production of biodegradable polymers through a combined anaerobic-aerobic process. *Journal of Chemical Technology and Biotechnology*, *84*(6), 901–908.
15. Bengtsson, S., Werker, A., & Welander, T. (2008). Production of polyhydroxyalkanoates by glycogen accumulating organisms treating a paper mill wastewater. *Water Science and Technology*, *58*(2), 323–330.
16. Bengtsson, S., Pisco, A.R., Reis P.C., Lemos, M. (2010). Production of polyhydroxyalkanoates from fermented sugar cane molasses by a mixed culture enriched in glycogen accumulating organisms. *J Biotechnol*. 145:253–263
17. Bhattacharya, S. K. (1996). Volatile solids reduction in two-phase and conventional anaerobic sludge digestion. *Water research* 30(5): 1041-1048.
18. Bouman R., Heffernan, B. (2016). AnMBR, anaerobic membrane bioreactor: from concept to full-scale and future outlook. Veolia Water Technologies Communications
19. Bugnicourt, E., Cinelli, P., Lazzeri, A., & Alvarez, V. (2014). Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging. *Express Polymer Letters*, *8*(11), 791–808.

20. Campanari, S., Silva, F.A., Bertin, L., Villano, M., Majone, M. (2014). Effect of the organic loading rate on the production of polyhydroxyalkanoates in a multi-stage process aimed at the valorization of olive oil mill wastewater. *Int J Biological Macromol.* 71:34–41

21. Carter, M., Fenedl, L., Malovrh, S., Logar, R.M., (2015) Biogas production from brewery spent grain enhanced by bioaugmentation with hydrolytic anaerobic bacteria. *Bioresource Technology*. 186:261-269

22. Cavaleiro, A.J. , Sousa, D.Z., Alves, M.M.. (2010). Methane production from oleate: Assessing the bioaugmentation potential of Syntrophomonas zehnderi. *Water Research*. 44(17): 4940-4947

23. Cecchi, F., P. Battistoni, P. Pavan, G. Fava and J. Mataalnarez. (1994). Anaerobic-Digestion of MSW (Organic Fraction of Municipal Solid Waste) and BNR (Biological Nutrients Removal) Process – A Possible Integration – Preliminary Results. *Water science and technology* 30(8): 65-72.

24. Chakrabarti, S. K. (1999). Biphasic biomethanation of wood-hydrolysate effluent. *Artificial Cells, Blood Substitutes, and Immobilization Biotechnology* 27(5-6): 461-467.

25. Chandra, R., H. Takeuchi and T. Hasegawa. (2012). Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. *Renewable sustainable energy reviews* 16(3): 1462-1476.

26. Chang, S. (2014). Anaerobic Membrane Bioreactors (AnMBR) for Wastewater Treatment. *Advances in Chemical Engineering and Science*, *4*(1), 56–61.

27. Chong, S., Sen, T. K., Kayaalp, A., & Ang, H. M. (2012). The performance enhancements of upflow anaerobic sludge blanket (UASB) reactors for domestic sludge treatment – A State-of-the-art review. *Water Research*, *46*(11), 3434–3470.

28. Cirne, D. G., Lehtomäki, A., Björnsson, L., & Blackall, L. L. (2007). Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops. *Journal of Applied Microbiology*, *103*(3), 516–527.

29. Coats, E. R., Loge, F. J., Wolcott, M. P., Englund, K., & McDonald, A. G. (2007). Synthesis of Polyhydroxyalkanoates in Municipal Wastewater Treatment. *Water Environment Research*, *79*(12), 2396–2403.

30. Cohen, A., J. Thiele and J. Zeikus. (1994). Pilot-Scale Anaerobic Treatment of Cheese Whey by the Substrate Shuttle Process. *Water science and technology* 30(12): 433-442.

31. Costa, J. C., S. G. Barbosa, M. M. Alves and D. Z. Sousa. (2012). Thermochemical pre- and biological co-treatments to improve hydrolysis and methane production from poultry litter. *Bioresource technology* 1111:41-147.

32. Da Silva, A. H., & Miranda, E. A. (2013). Adsorption/desorption of organic acids onto different adsorbents for their recovery from fermentation broths. *Journal of Chemical and Engineering Data*, *58*(6), 1454–1463.

33. Davidsson, A., J. Wawrzynczyk, O. Norrlow and J. L. Jansen. (2007). Strategies for enzyme dosing to enhance anaerobic digestion of sewage sludge. *Journal of residuals science and technology* 4(1): 1-7.

34. Demirer, G. and S. Chen. (2005). Two-phase anaerobic digestion of unscreened dairy manure. *Process biochemistry* 40(11): 3542-3549.

35. De Vrieze, J., Hennebel, T., Boon, N., & Verstraete, W. (2012). Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. *Bioresource Technology*, *112*, 1–9.

36. Diekert, G., & Wohlfarth, G. (1994). Metabolism of homoacetogens. In *Antonie van Leeuwenhoek* (pp. 66–209). Journal of Microbiology.

37. Dionisi, D., Majone, M., Vallini, G., Di Gregorio, S. Beccari, M. (2006). Effect of the applied organic load rate on biodegradable polymer production by mixed microbial cultures in a sequencing batch reactor. *Biotechnol Bioeng*. 93:76–88

38. Duran, M., N. Tepe, D. Yurtsever, V. Punzi and C. Bruno. (2006). Bioaugmenting anaerobic digestion of biosolids with selected strains of Bacillus, Pseudomonas, and Actinomycetes species for increased methanogenesis and odor control. *Applied Microbiology and Biotechnology* 73(4): 960-966.

39. Duque, A.F. Oliveira, A.R. Carmo, C., Gouveia, I., Pardelha, F. et al. (2014).Response of a three-stage process for PHA production by mixed microbial cultures to feedstock shift: impact on polymer composition. *New Biotechnol*. 31:276–288

40. Dvořák, Lukáš, Marcel Gómez, Jan Dolina, and A. Č. (2016). Anaerobic membrane bioreactors—a mini review with emphasis on industrial wastewater treatment: applications, limitations and perspectives. *Desalination and Water Treatment*, 3994:1–15.

41. Eastman, J. a, & Ferguson, J. F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *Journal of the Water Pollution Control Federation*, *53*(3), 352–366.

42. El Fantroussi, S. and S. Agathos. (2005). Is bioaugmentation a feasible strategy for pollutant removal and site remediation? *Current opinion in microbiology* 8(3): 268-275.

43. Fatone, F., Frison, N., Katsou, E., Malamis, S. (2014). Integrating the selection of PHA storing biomass and nitrogen removal via nitrite for the treatment of the sludge reject water. *Wastewater and Biosolids Treatment and Reuse: Bridging Modeling and Experimental Studies*. Eds, ECI Symposium Series

44. Fotidis, I. A., Wang, H:, Fiedel, N. R., Luo, G., Karakashev, D. B., Angelidaki, I. (2014). Bioaugmentation as a solution to increase methane production from an ammonia-rich substrate. *Environmental Science and Technology*. 48:7669-7676

45. Frison N, Katsou E, Malamis S, Oehmen A, Fatone F. (2015) Development of a Novel Process Integrating the Treatment of Sludge Reject Water and the Production of Polyhydroxyalkanoates (PHAs). Environmental Science and Technology. 49(18):10877-85

46. Fuchs, W., Binder, H., Mavrias, G., & Braun, R. (2003). Anaerobic treatment of wastewater with high organic content using a stirred tank reactor coupled with a membrane filtration unit. *Water Research*, *37*(4), 902–908.

47. Gharsallah, N. (1994). Influence of dilution and phase separation on the anaerobic digestion of olive oil mill wastewaters. *Bioprocess Engineering* 1029.

48. Ghosh, S. (1987). Improved sludge gasification by two-phase anaerobic digestion. *Journal of Environmental Engineering* 116(6): 1265-1284.

49. Ghosh, S. (1985). Methane production from industrial wastes by two-phase anaerobic digestion. *Water research* 19(9): 1083-1088.

50. Ghyoot, W. R., & Verstraete, W. H. (1997). Coupling membrane filtration to anaerobic primary sludge digestion. *Environmental Technology*, *18*(6), 569–580.

51. Gianfreda, L. and M. Rao. (2004). Potential of extra cellular enzymes in remediation of polluted soils: a review. *Enzyme and microbial technology* 35(4): 339-354.

52. Grethlein, H. E. (1978). Anaerobic Digestion and membrane separation of Domestic Wastewater. *Water Pollution Control Federation*, *50*(4), 754–763.

53. Guiot, B. Tartakovsky, M. Lanthier, M. Levesque and M. Manuel. (2002). Strategies for augmenting the pentachlorophenol degradation potential of UASB anaerobic granules. *Water science and technology* 45(10): 35-41.

54. Gujer, W., & Zehnder, A. J. B. (1983). Conversion processes in anaerobic digestion. *Water Science and Technolgy*, *15*(8–9), 127–167.

55. Head, M. and J. Oleszkiewicz. (2005). Bioaugmentation with nitrifying bacteria acclimated to different temperatures. *Journal of Environmental Engineering* 131(7): 1046-1051.

56. Herrero, M., & Stuckey, D. C. (2015). Bioaugmentation and its application in wastewater treatment: A review. *Chemosphere*, *140*, 119–128.

57. Hooper, R. and J. Li. (1996). Summary of the factors critical to the commercial application of bioenergy technologies. *Biomass bioenergy* 11(6): 469-474.

58. Hu, A. Y., & Stuckey, D. C. (2007). Activated Carbon Addition to a Submerged Anaerobic Membrane Bioreactor: Effect on Performance, Transmembrane Pressure, and Flux. *Journal of Environmental Engineering*, *133*(1), 73–80.

59. Ivanov, V., Stabnikov, V., Ahmed, Z., Dobrenko, S., & Saliuk, A. (2014). Production and applications of crude polyhydroxyalkanoate-containing bioplastic from the organic fraction of municipal solid waste. *International Journal of Environmental Science and Technology*, *12*(2), 725–738.

60. Johnson, K., Jiang, Y., Kleerebezem , R., Muyzer, G., van Loosdrecht, MCM. (2009) Enrichment of a mixed bacterial culture with a high polyhydroxyalkanoate storage capacity. Biomacromolecules. 10:670-676

61. Jiang, Y., Marang, L., Tamis, J., van Loosdrecht, M. C. M., Dijkman, H., & Kleerebezem, R. (2012). Waste to resource: Converting paper mill wastewater to bioplastic. *Water Research*, *46*(17), 5517–5530.

62. Ketheesan, B., & Stuckey, D. C. (2015). Effects of Hydraulic/Organic Shock/Transient Loads in Anaerobic Wastewater Treatment: A Review. *Critical Reviews in Environmental Science and Technology*, *45*(24), 2693–2727.

63. Khanal, S.K., Huang, J.C., (2005) Effect of high influent sulfate on anaerobic wastewater treatment. *Water Environ. Res*. 77, 3037–3046.

64. Kim, J. O., & Somiya, I. (2001). Effect of hydraulic loading rate on acidogenesis in a membrane-coupled anaerobic VFAs fermenter. *Environmental Technology*, *22*(1), 91–99.

65. Korkakaki, E., Mulders, M., Veeken, A., Rozendal, R., van Loosdrecht, M. C. M., & Kleerebezem, R. (2016). PHA production from the organic fraction of municipal solid waste (OFMSW): Overcoming the inhibitory matrix. *Water Research*.

66. Kugel, G., E. Zingler and G. Hellifeier. (1992). Upgrading of 100,000 PE Goch Sewage-Treatment Plant – Design of 2-Stage Sewage-Treatment and Anaerobic Thermophilic/Mesophilic Sludge-Stabilization. *Water science and technology* 25(4-5): 331-337.

67. Lee, S. Y. (1996). Bacterial Polydroxyalkanoates. *Biotechnology and Bioengineering*, *49*, 1–14.

68. Lee, S. M., Jung, J. Y., & Chung, Y. C. (2001). Novel method for enhancing permeate flux of submerged membrane system in two-phase anaerobic reactor. *Water Research*.

69. Liao, B.-Q., Kraemer, J. T., & Bagley, D. M. (2006). *Anaerobic Membrane Bioreactors: Applications and Research Directions*. *Critical Reviews in Environmental Science and Technology* (Vol. 36).

70. Lin, H., Peng, W., Zhang, M., Chen, J., Hong, H., & Zhang, Y. (2013). A review on anaerobic membrane bioreactors: Applications, membrane fouling and future perspectives. *Desalination*, *314*, 169–188.

71. Luengo, J.M., Garcia, B., Sandoval, A., Naharro, G., Olivera, E.R. (2003). Bioplastics from microorganisms. *Current Opinion in Microbiology*. 6:251-260

72. Majone, M., Beccari, M, Di Gregorio, S, Dionisi, D, Vallini, G. (2005). Enrichment of activated sludge in a sequencing batch reactor for polyhydroxyalkanoate production. Water Science and Technology. 54(1):119-128

73. Martin, I., Pidou, M., Soares, A., Judd, S., & Jefferson, B. (2011). Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment. *Environmental Technology*, *32*(9), 921–932.

74. Martin-Ryals, A., Schideman, L., Li, P., Wilkinson, H., Wagner, R. (2015). Improving anaerobic digestion of a cellulosic waste via routine bioaugmentation with cellulolytic microorganisms. *Bioresource Technology*, 189:62-70

75. Massey, M. L. (1978). Phase separation of anaerobic stabilization by kinetic controls. *Journal - Water Pollution Control Federation* 50(9): 2204-2222.

76. Meabe, E., Déléris, S., Soroa, S., & Sancho, L. (2013). Performance of anaerobic membrane bioreactor for sewage sludge treatment: Mesophilic and thermophilic processes. *Journal of Membrane Science*, *446*, 26–33.

77. Meng, F., Chae, S. R., Drews, A., Kraume, M., Shin, H. S., & Yang, F. (2009). Recent advances in membrane bioreactors (MBRs): Membrane fouling and membrane material. *Water Research*, *43*(6), 1489–1512.

78. Mengmeng, C., Hong, C., Qingliang, Z., Shirley, S. N., Jie, R. (2009) Optimal production of polyhydroxyalkanoates (PHA) in activated sludge fed by volatile fatty acids (VFAs) generated from alkaline excess sludge fermentation. Bioresource Technology 100:1399-1405

79. Metcalf and Eddy Inc. (2003). *Wastewater Engineering: Treatment and Resue* (4th ed.)

80. Mladenovska, Z., Y. Ishoy, A. Mandiralioglu, P. Westermann and Ahring B. K. (2001). Bioaugmentation of a mesophilic biogas reactor by anaerobic xylanolytic- and cellulolytic bacteria. In *Proceedings of the 9th World Congress of Anaerobic Digestion,* 183. ed. Antwerp, Belgium

81. Morgan-Sagastume, F., Hjort, M., Cirne, D., Grardin, F., Lacroix, S., Gaval, G.,Werker, A. (2015). Integrated production of polyhydroxyalkanoates (PHAs) with municipal wastewater and sludge treatment at pilot scale. *Bioresource Technology*, *181*, 78–89.

82. Morgan-Sagastume, F., Karlsson, A., Johansson, P., Pratt, S., Boon, N. (2010) Production of polyhydroxyalkanoates in open, mixed cultures, from a waste sludge stream containing high levels of soluble organics, nitrogen, and phosphorus. *Water Research*. 44:5196-211

83. Muller, V., Frerichs, J. (2013). Acetogenic Bacteria. *eLS*

84. Mumme, J., B. Linke and R. Toelle. (2010). Novel upflow anaerobic solid-state (UASS) reactor. *Bioresource technology* 101(2): 592-599

85. Murata, M., Kimuro, H., Kanekuni, N., Ohkuma, N., Ogasawara, H., & Fujioka, T. (1994). Small-scale sewage plant experiment by pretreatment and methanization of suspended solids. *Desalination*, *98*(1–3), 217–224.

86. Neumann, L. and P. Scherer. (2011). Impact of bioaugmentation by compost on the performance and ecology of an anaerobic digester fed with energy crops. *Bioresource technology* 102(3): 2931-2935.

87. Ng, W. J. (1985). Two-phase anaerobic treatment kinetics of palm oil wastes. *Water research* 19(5): 667-669.

88. Nielsen, H., Z. Mladenovska and B. Ahring. (2007). Bioaugmentation of a two-stage thermophilic (68 degrees C/55 degrees C) anaerobic digestion concept for improvement of the methane yield from cattle manure. *Biotechnology and bioengineering* 97(6): 1638-1643.

89. Norddahl, B., & Rohold, L. (2000). The BiorekR concept for the conversion of organic effluent to energy, concentrated fertilizer and potable water.

90. Padmasiri, S. I., Zhang, J., Fitch, M., Norddahl, B., Morgenroth, E., & Raskin, L. (2007). Methanogenic population dynamics and performance of an anaerobic membrane bioreactor (AnMBR) treating swine manure under high shear conditions. *Water Research*, *41*(1), 134–144.

91. Parawira, W. (2012). Enzyme research and applications in biotechnological intensification of biogas production. *Critical reviews in biotechnology* 32(2): 172-186.

92. Park, C., C. Lee, S. Kim, Y. Chen and H. Chase. (2005). Upgrading of anaerobic digestion by incorporating two different hydrolysis processes. *Journal of bioscience and bioengineering* 100(2): 164-167.

93. Park, H., Choo, K.-H., & Lee, C.-H. (1999). Flux Enhancement with Powdered Activated Carbon Addition in the Membrane Anaerobic Bioreactor. *Separation Science and Technology*, *34*(14), 2781–2792.

94. Parmar, N., A. Singh and O. Ward. (2001). Enzyme treatment to reduce solids and improve settling of sewage sludge. *Journal of industrial microbiology biotechnology* 26(6): 383-386.

95. Pavan, P., P. Battistoni, F. Cecchi and J. Mata Alvarez. (2000). Two-phase anaerobic digestion of source sorted OFMSW (organic fraction of municipal solid waste): performance and kinetic study. *Water science and technology* 41(3): 111-118.

96. Peng, X, Börner, R.A, Nges I.A, Liu, J. (2014). Impact of bioaugmentation on biochemical methane potential for wheat straw with addition of Clostridium cellulolyticum. *Bioresource Technology.*152:567-571

97. Pierkiel, A., & Lanting, J. (2005). Membrane-coupled anaerobic digestion of municipal sewage sludge. *Water Science & Technology*, *52*(1–2), 253–258.

98. Pillay, V. L., Townsend, B., & Buckley, C. A. (1994). Improving the performance of anaerobic digesters at wastewater treatment works: the coupled cross-flow microfiltration/digester process. *Water Science & Technology*, *30*(12), 329–337.

99. Prashanth, S., Kumar, P., Mehrotra, I. (2006). Anaerobic degradability: effect of particulate COD. Journal of Environmental Engineering. 132(4):488-496

100. Raynal, J., J. Delgenes and R. Moletta. (1998) Two-phase anaerobic digestion of solid wastes by a multiple liquefaction reactors process. *Bioresource technology* 65(1-2): 97-103.

101. Rebecchi, S., Pinelli, D., Bertin, L., Zama, F., Fava, F., & Frascari, D. (2016). Volatile fatty acids recovery from the effluent of an acidogenic digestion process fed with grape pomace by adsorption on ion exchange resins. *Chemical Engineering Journal*, *306*, 629–639.

102. Reddy, M.V.,Mohan., S.V. (2012). Effect of substrate load and nutrients concentration on the polyhydroxyalkanoates (PHA) production using mixed consortia through wastewater treatment. *Biores Technol*, 114:573–582

103. Ritmann, B. E. and R. Whiteman. (1994). Bioaugmentation: A coming of age. *Water Quality International* 112.

104. Roman, H., J. Burgess and B. Pletschke. (2006). Enzyme treatment to decrease solids and improve digestion of primary sewage sludge. *African journal of biotechnology* 5(10): 963-967.

105. Romano, R., R. Zhang, S. Teter and J. McGarvey. (2009). The effect of enzyme addition on anaerobic digestion of Jose Tall Wheat Grass. *Bioresource technology* 100(20): 4564-4571.

106. Rulkens, W. (2008). Sewage Sludge as a Biomass Resource for the Production of Energy: Overview and Assessment of the Various Options. Energy and Fuels. 22(1):9-15

107. Saravanane, R., D. Murthy and K. Krishnaiah. (2001). Bioaugmentation and anaerobic treatment of pharmaceutical effluent in fluidized bed reactor. *Journal of environmental science and health. Part A, Toxic/hazardous substances environmental engineering* 36(5): 779-791.

108. Satoh, H., S. Okabe, Y. Yamaguchi and Y. Watanabe. (2003). Evaluation of the impact of bioaugmentation and biostimulation by in situ hybridization and microelectrode. *Water research* 37(9): 2206-2216.

109. Schauer Gimenez, A., D. Zitomer, J. Maki and C. Struble. (2010). Bioaugmentation for improved recovery of anaerobic digesters after toxicant exposure. *Water research* 44(12): 3555-3564.

110. Serafim, L. S., Lemos, P. C., Albuquerque, M. G. E., & Reis, M. A. M. (2008). Strategies for PHA production by mixed cultures and renewable waste materials. *Applied Microbiology and Biotechnology*, *81*(4), 615–628.

111. Setiadi, T., Trianto, A., Aznury, M., & Pancoro, A. (2015). Production of polyhydroxyalkanoate (PHA) by ralstonia eutropha JMP 134 with volatile fatty acids from palm oil mill effluent as precursors. *Water Science and Technology*, *72*(11), 1889–1895.

112. Shah, F.A, Mahmood, Q., Shah, M.M, Pervez, A., and Asad, S.A. (2014). Microbial Ecology of Anaerobic Digesters: The Key Players of Anaerobiosis. *The Scientific World Journal*, 183752, 21

113. Smith, A. L., Skerlosab, S. J., Raskin, L. (2015). Anaerobic membrane bioreactor treatment of domestic wastewater at psychrophilic temperatures ranging from 15 °C to 3 °C. Environmental Science: Water Research & Technology. 1:56-64

114. Stams, A., Plugge, C., Bok, F., Houten, B., Lens, P., Dijkman, H., Weijma, J., (2005). Metabolic interactions in methanogenic and sulfate-reducing bioreactors. *Water Sci. Technol*. 52, 13–20.

115. Stuckey, D. C. (2012). Recent developments in anaerobic membrane reactors. *Bioresource Technology*, *122*, 137–148.

116. Tale, V.P., Maki, J.S., Zitomer, D.H. (2015). Bioaugmentation of overloaded anaerobic digesters restores function and archaeal community. *Water Research*. 70:138-147

117. Tamis, J., Lužkov, K., Jiang, Y., Loosdrecht, M. C. M. van, & Kleerebezem, R. (2014). Enrichment of Plasticicumulans acidivorans at pilot-scale for PHA production on industrial wastewater. *Journal of Biotechnology*, *192*(Part A), 161–169.

118. Teo, C. W., & Wong, P. C. Y. (2014). Enzyme augmentation of an anaerobic membrane bioreactor treating sewage containing organic particulates. *Water Research*, *48*, 335–344.

119. Trzcinski, A. P., & Stuckey, D. C. (2009). Continuous treatment of the organic fraction of municipal solid waste in an anaerobic two-stage membrane process with liquid recycle. *Water Research*, *43*(9), 2449–2462.

120. Tyagi, V. K., & Lo, S. L. (2013). Sludge: A waste or renewable source for energy and resources recovery? *Renewable and Sustainable Energy Reviews*, *25*(71), 708–728.

121. Valentino, F., Morgan-Sagastume, F., Campanari, S., Villano, M., Werker, A., & Majone, M. (2017). Carbon recovery from wastewater through bioconversion into biodegradable polymers. *New Biotechnology*.

122. Valentino, F. Beccari, M. Fraraccio, S. Zanaroli, G. Majone. M. (2014). Feed frequency in a Sequencing Batch Reactor strongly affects the production of polyhydroxyalkanoates (PHAs) from volatile fatty acids. *New Biotechnol*, 31:264–275

123. Valentino, F., Karabegovic, L., Majone, M., Morgan-Sagastume, F., & Werker, A. (2015). Polyhydroxyalkanoate (PHA) storage within a mixed-culture biomass with simultaneous growth as a function of accumulation substrate nitrogen and phosphorus levels. *Water Research*, *77*, 49–63.

124. Van Lier, J., S. Rebac and G. Lettinga. (1997). High-rate anaerobic wastewater treatment under psychrophilic and thermophilic conditions. *Water science and technology* 35(10): 199-206.

125. Van Limbergen, H., E. Top and W. Verstraete. (1998). Bioaugmentation in activated sludge: current features and future perspectives. *Applied Microbiology and Biotechnology* 50(1): 16-23.

126. Van Loosdrecht, M. C. M., Pot, M. A., & Heijnen, J. J. (1997). Importance of bacterial storage polymers in bioprocesses. *Water Science and Technology*.

127. Venkiteshwaran, K., Milferstedt, K., Hamelin, J., & Zitomer, D. H. (2016). Anaerobic digester bioaugmentation influences quasi steady state performance and microbial community. *Water Research*, *104*, 128–136.

128. Villano, M., Lampis, S., Valentino, F., Vallini, G., Majone, M. et al. (2010). Effect of hydraulic and organic loads in Sequencing Batch Reactor on microbial ecology of activated sludge and storage of polyhydroxyalkanoates. *Chem Eng Transactions*. 20:187–192

129. Villano, M., Beccari, M., Dionisi, D., Lampis, S., Miccheli, A.,et al. (2010). Effect of pH on the production of bacterial polyhydroxyalkanoates by mixed cultures enriched under periodic feeding. *Process Biochem*. 45:714–723

130. Villano, M., Valentino, F., Barbetta, A., Martino, L., Scandola, M., & Majone, M. (2014). Polyhydroxyalkanoates production with mixed microbial cultures: From culture selection to polymer recovery in a high-rate continuous process. *New Biotechnology*, *31*(4), 289–296.

131. Visvanathan, C., & Abeynayaka, A. (2012). Developments and future potentials of anaerobic membrane bioreactors (AnMBRs). *Membrane Water Treatment*, *3*(1), 1–23.

132. Walker, M., Banks, C. J., & Heaven, S. (2009). Development of a coarse membrane bioreactor for two-stage anaerobic digestion of biodegradable municipal solid waste. *Water Science and Technology*, *59*(4), 729–735.

133. Wawrzynczyk, J. (2003). Alternative method for sludge reduction using commercial enzymes. *In: Aqua enviro technology transfer: eighth CLWEM/aqua enviro European biosolids and organic residuals conference*.

134. Weiss, S., M. Tauber, W. Somitsch, R. Meincke and H. Muella. (2010). Enhancement of biogas production by addition of hemicellulolytic bacteria immobilized on activated zeolite. *Water research* 44(6): 1970-1980.

135. Wen, Q., Chen, Z., Tian, T., Chen. W. (2010). Effects of phosphorus and nitrogen limitation on PHA production in activated sludge. *J Environ Sci*. 22:1602–1607

136. WERF (2008). Wastewater sludge: A new resource for alternative energy. www.wateronline.com

137. Wheaton, R. M., Lefevre, L. J., (2000). DOWEX Ion Exchange Resins, Fundamentals of Ion Exchange. Dow Chemical U.S.A.

138. Xu, M., Wen, X., Huang, X., & Li, Y. (2010). Membrane Fouling Control in an Anaerobic Membrane Bioreactor Coupled with Online Ultrasound Equipment for Digestion of Waste Activated Sludge. *Separation Science and Technology*, *45*(October 2014), 941–947.

139. Yadvika, Santosh, Sreekrishnan, T.R., Kohli, S., Rana, V. (2004). Enhancement of biogas production from solid substrates using different techniques – a review. Bioresource Technology. 95:1-10

140. Yeoh, B. (1997). Two-phase anaerobic treatment of cane-molasses alcohol stillage. *Water science and technology* 36(6-7): 441-448.

141. Yoo, R., Kim, J., Mccarty, P. L., & Bae, J. (2012). Bioresource Technology Anaerobic treatment of municipal wastewater with a staged anaerobic fluidized membrane bioreactor (SAF-MBR) system. *Bioresource Technology*, *120*, 133–139.

142. Yushina, Y. and J. Hasegawa. (1994). Process Performance Comparison of Membrane Introduced Anaerobic-Digestion Using Food-Industry Waste-Water. *Desalination* 98(1-3): 413-421.

143. Zhang, T. C. (1991). Comparison of one-phase and two-phase anaerobic digestion processes in characteristics of substrate degradation and bacterial population levels. *Water science and technology* 23(7-9): 1157-1166.

144. Zhang, J., Guo, R.B., Qiu, Y.L., Qiao, J.T., Yuan, X.Z., Shi, X.S. (2015). Bioaugmentation with an acetate-type fermentation bacterium Acetobacteroides hydrogenigenes improves methane production from corn straw. *Bioresource Technology*. 186:286-293

145. Oehmen, A., Pinto, FV., Silva, V., Albuquerque, MAM. (2014). The impact of pH control on the volumetric productivity of mixed culture PHA production from fermented molasses. Eng. Life Sci. 14:143-152

CHAPTER 3: BIOAUGMENTATION TO IMPROVE PERFORMANCE OF A TWO-PHASE ANMBR TREATING SEWAGE SLUDGE

Abstract

Incorporation of bioaugmentation in the acid-phase of a two-phase anaerobic membrane bioreactor (AnMBR) system treating primary sludge was investigated as a means for targeting and improving substrate hydrolysis and acetogenesis. Bioaugmentation was carried out using a proprietary bioculture blend containing a mixture of hydrolytic, acidogenic, and acetogenic microorganisms. This mixture was added both on its own and in combination with recycled anaerobic sludge from the methane-phase of the AnMBR. These bioaugmentation strategies increased average percent hydrolysis by 25-38%, and increased average acid-phase acetic acid generation by 31-52% compared to operation without bioaugmentation. These benefits led to subsequent increases in average methane production (10-13%) and greater average overall solids reduction by 25-55%. Finally, microbial community analysis using 16S Illumina MiSeq generated sequences confirmed increased relative abundance of bioaugmented microorganism including *Acetobacter* and *Syntrophomonas* species. Overall, bioaugmentation was found to improve conversion of primary sludge to methane by shifting the microbial community towards one better suited for hydrolysis and acetogenesis.

Introduction

Anaerobic digestion is commonly used as a means for treating the solids fraction of domestic wastewater, namely sewage sludge. In this process, organic material is converted into biogas, a mixture of methane and carbon dioxide, by a consortium of facultative and anaerobic microorganisms working synergistically through a series of four phases: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Compared to aerobic processes, anaerobic treatment offers the benefits of energy recovery as methane, reduced solids production, and elimination of the cost and energy requirements associated with aeration (Chong et al., 2012; Teo et al., 2014). However, some disadvantages of anaerobic treatment, relative to aerobic treatment, include poorer effluent quality, which leads to additional post treatment requirements, and longer retention times (30-50 days), which equates to larger reactor volumes resulting in increased capital cost and land requirements (Chong et al., 2012; Teo et al., 2014; Yadvika et al., 2004).

Anaerobic membrane bioreactors (AnMBR) offer a potential solution to these problems, via the integration of membrane filtration with anaerobic treatment.

In an AnMBR, a micro- or ultra-filtration membrane component is integrated with the anaerobic digester in order to decouple the liquid and solid retention times. In effect, the liquid fraction of the waste stream is able to pass through the membrane and exit the system quickly, reducing volume requirements, while the solids fraction is retained by the membrane providing sufficient time for degradation. Besides reducing reactor volume, this also leads to the benefits of increased methane production, reduced waste sludge production, and production of a solids free effluent with potential for water reuse. However, a drawback to AnMBRs is the fact that significant solids accumulation in an AnMBR can lead to impediment of the treatment processes contributing to inhibition of methanogenic activity, process instability, and/or membrane fouling (Jeison et al., 2008; Teo et al., 2014). Prashanth et al., (2006) found that increasing particular solids concentration in synthetic wastewater led to a decline in methane production rates. Considering that particulate solids can account for 30-70% of the chemical oxygen demand (COD) of wastewater, it is therefore critical that effective particulate solids reduction is achieved in order to maintain optimum AnMBR performance.

For anaerobic treatment of waste streams with a high concentration of particular solids, including sewage sludge, hydrolysis has been recognized as the major-rate limiting step (Eastman & Ferguson, 1981; Teo et al., 2014, Mumme et al., 2010; Park et al., 2005). Therefore, targeting and improving rates of hydrolysis can reduce solids accumulation and enhance the overall conversion of sewage sludge to methane. A potential means for achieving is this through bioaugmentation with hydrolytic microorganisms. In general, bioaugmentation, or the addition of specific microorganisms to a system in order to correct of enhance a desired process or activity, has been recognized as a promising strategy for solving various problems associated with wastewater treatment, including solids removal (Herrero and Stuckey, 2015). Several studies have shown bioaugmentation with hydrolytic microorganisms to significantly increase solids reduction and methane production from a variety of substrates (Angelidaki & Ahring, 2000; Cirne et al., 2006; Costa et al., 2012; Martin-Ryals et al., 2015; Mladenovska et al., 2001; Nielsen et al., 2007; Weiss et al., 2010), however, is many cases, these benefits were not sustained over time to due to washout of the bioaugmented microorganisms or their inability to compete within the indigenous microbial community (Mladenovska et al., 2001; Nielsen et al.,

2007). In this respect, bioaugmentation and AnMBR technology complement each other, as the membrane can provide a means for retaining bioaugmented microorganisms in the system (Herrero & Stuckey, 2015).

In addition to hydrolytic bacteria, bioaugmentation with acetic acid producing bacteria or acetogens could also benefit system performance. Acetate is a key substrate for methanogenesis with approximately 70% of methane production in anaerobic treatment processes being produced from the reduction of acetate (Shah et al., 2014). In anaerobic treatment, acetate is produced by either acetic acid producing bacteria though fermentation of soluble intermediates, or by acetogens via anaerobic oxidation of volatile fatty acids or the reduction of CO_2 with H_2 (Diekert & Wohlfarth, 1994; Miron et al., 2000; Shah et al., 2014). Accumulation of volatile fatty acids can be detrimental to process performance due to the associated decrease in pH which can inhibit methanogenesis (Goux et al., 2015). Thus, bioaugmentation with acetogens and acetic acid producing bacteria could benefit process performance by both increasing the availability of acetate for methanogenesis and by reducing the accumulation of volatile fatty acids and other soluble intermediates that could lead to process inhibition.

The objective of this study was to investigate bioaugmentation as a means for targeting and improving hydrolysis and acetic acid production, in a two-phase AnMBR system treating primary sewage sludge at mesophilic temperature. Bioaugmentation was applied daily to the acid-phase, using two different strategies. The first consisted of the addition of a proprietary bioculture mixture along with recycling of anaerobic sludge from the methane-phase. The second consisted of adding only the proprietary bioculture. The impact of bioaugmentation on substrate hydrolysis and acetic acid production, as indicated by soluble COD (sCOD) generation and acid-phase volatile fatty acid (VFA) generation was evaluated as well as the subsequent effects on methane production and overall solids reduction. Finally, Illumina MiSeq sequencing was used to evaluate changes in the microbial community structure as a result of bioaugmentation.

Materials and Methods

Primary Sludge Substrate

The substrate for this study was primary sludge collected from the Urbana-Champaign Sanitary (UCSD) Northeast Wastewater Treatment Plant. Primary sludge was collected at four different time points over the course of this study. Each time 150-200 L of primary sludge was collected in 5 gallon plastic buckets and stored at 4°C until use. Table 3.1 provides characteristics of the four batches of primary sludge collected during this study. Elemental carbon, hydrogen, and nitrogen (CHN) analysis of the primary sludge was conducted by the University of Illinois at Urbana-Champaign (UIUC) Microanalysis Lab using a CHN analyzer (Exeter Analytical, Inc. CE-440). The percentage of oxygen was determined by subtracting the percentage of the other three elements and the ash content from 100. Theoretical maximum methane yield for the primary sludge substrate was then calculated based on elemental make-up and Boyle´s equation (Nielfa et al, 2014):

$$C_aH_bO_cN_d + \frac{(4a-b-2c+3d)}{4\,H_2O} = \frac{(4a+b-2c-3d)}{8\,CH_4} + \frac{(4a+b-2c-3d)}{8\,CO_2} dNH_3$$

Table 3.1. Characteristics of primary sludge substrate.

Parameter	Units	Batch 1	Batch 2	Batch 3	Batch 4
Period of Use	Days	0 - 302	303 - 485	486 - 666	667-947
Total COD	mg/L	43,861 ±5,378	32,206 ±10,177	31,703 ±10,235	36,891 ±11684
Soluble COD	mg/L	5,053 ±1,189	5,342 ±1,042	6,249 ±1,497	6,464 ±1300
Total Solids	g/L	34.02 ±0.88	26.23 ±4.08	24.78 ±5.58	24.2 ±3.3
Volatile Solids	g/L	27.88 ±0.10	22.57 ±3.90	19.30 ±3.05	19.5 ±3.4
C:H:N:O *(by mass)*		44:6:3:30	39:5:3:28	43:6:5:23	43:6:3:31
Theoretical Max. CH_4	ml/g VS	458.6 ±23.2	424.3.7 ±8.8	469.8 ±15.9	436.0 ±15.6
pH		5.34	5.55	5.35	5.43

Proprietary Bioculture used for Bioaugmentation

A dry bioculture blend provided by Microbial Energy Systems, Inc. was utilized for bioaugmentation in this study. The bioculture consisted of a 1:1:1 mixture (VS basis) of three bioculture mixtures previously developed to augment anaerobic digestion of citrus, hog manure, and cellulosic wastes, and presumed to contain a mixture of hydrolytic, acidogenic, and acetogenic bacteria. The bioculture consisted of dehydrated bacteria attached to a cornmeal

medium with a total volatile solids content of 95 ± 0.02%. The bioculture was stored in an airtight container at 4°C between applications. Prior to this study, batch testing of the proprietary bioculture to evaluate its benefit on sCOD and VFA generation from primary sludge was conducted (results not shown). In this batch test, a deactivated bioculture control condition was also investigated in order to verify that the observed benefits to sCOD and VFA generation was in fact the result of microbial activity in the bioculture.

Set-up and Operation of Continuous Two-phase AnMBR System

A bench-scale continuous two-phase AnMBR system was set up to study the effects of bioaugmentation in the acid-phase on system performance. A photo of the actual two-phase AnMBR system is shown as Figure 3.1, and a schematic representation is shown in Figure 3.2. The acid-phase reactor consisted of a 2.5 L (working volume of 1.5 L) spinner flask (Bellco) with heating and mixing provided by a heated magnetic stir-plate combined with a magnetic stir-bar attached to an impeller inside the reactor. The methane-phase reactor consisted of a 14 L (working volume 12 L) New Brunswick BioFlo 115 bioreactor. The BioFlo control unit provided mixing and control of temperature, pH and liquid levels inside the methane-phase reactor. Default settings for mixing and pH were 120 RPM and 7.4, respectively. Automatic additions of 10M NaOH via the BioFlo control unit were made to maintain pH in the methane-phase reactor. Temperature in both the acid- and methane-phase was maintained at mesophilic conditions (37°C). A wet tip gas meter (www.wettipgasmeter.com) was installed to measure methane production.

At the start of operations, the acid-phase was seeded with a 1:4 dilution of the primary sludge substrate in water. The methane-phase was initially seeded with mesophilic anaerobic sludge collected from the primary anaerobic digester of the UCSD Northeast Wastewater Treatment Plant. During start-up of the AnMBR system, a relatively low total organic loading rate (OLR) of 0.4 g COD/L d-1 was applied. The OLR was gradually increased 2.4 g COD/L d^{-1} by Day 87. A slight decrease in OLR, from 2.4-1.8 g COD/L d^{-1} occurred over the course of the study due to variation in the COD content of the latter batches of primary sludge that were used during this study.

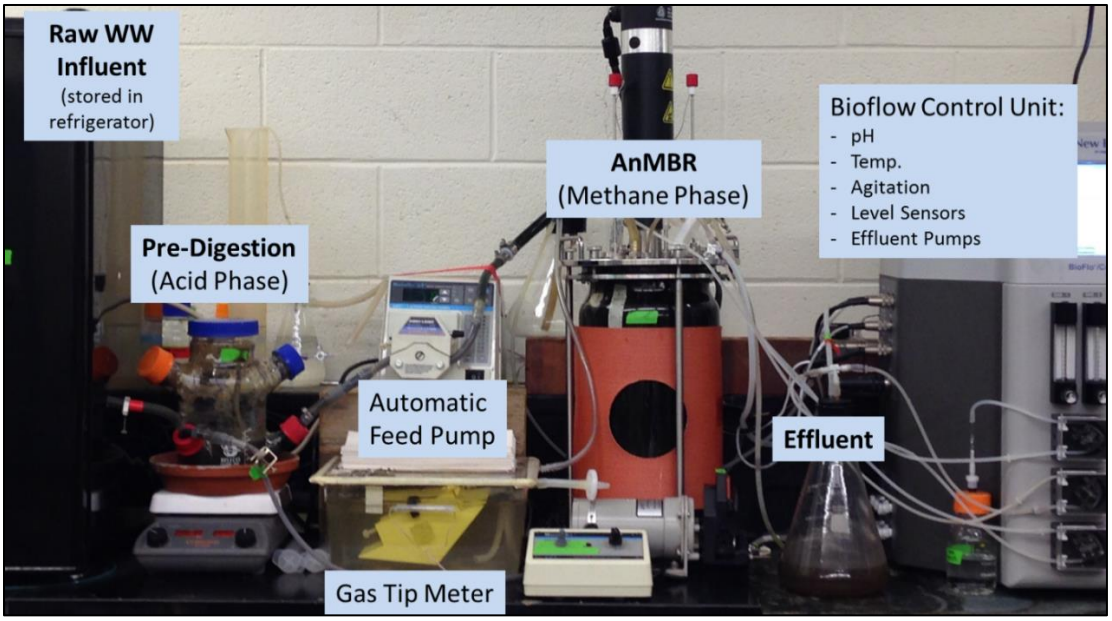


Figure 3.1. Photo of two-phase continuous AnMBR system.

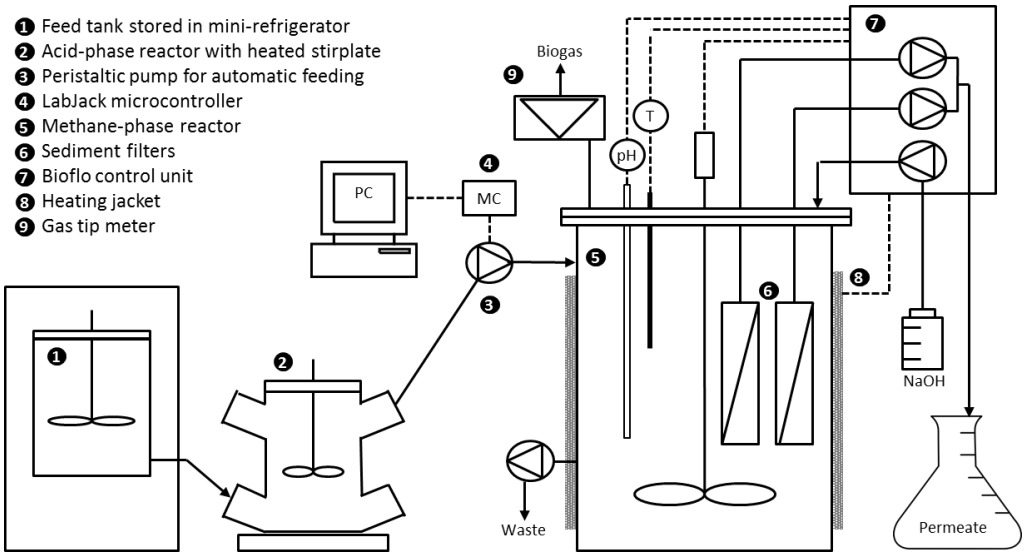


Figure 3.2. Schematic of two-phase continuous AnMBR system.

HRT and SRT in the acid-phase were maintained at 2 days. Average HRT in the methane-phase was 16 days, with an average SRT of 30 days for the majority of the study. Automatic feeding in the system was carried out via a computer Python script used to command a Labjack U3 DAC which controlled a Masterflex LS 07523-40 pump that intermittently pumped liquid from the acid-phase reactor to the methane-phase reactor to maintain the desired flow rate of 0.75 L/day. Upon pumping material between the acid- and methane-phase reactors, new

influent was drawn into the acid-phase reactor via gravity from a stirred and refrigerated influent storage tank (4^{o}C). The Bioflo unit controlled the pumping of effluent out of the system through the microfiltration membranes inside the methane-phase reactor.

For the majority of operation, two 10 µm pore size, 0.11 m2 cylindrical Omnifilter RS14-DS sediment filter cartridges served as the submerged membrane filtration component of the AnMBR system, as shown in Figure 3.3. To accommodate their placement in the methane-phase reactor, the filters were cut in half length wise and manifolded together. The filters had a final combined surface area of 0.258 m^2. From Days 131 to 196 a custom-built 0.2 µm pore size, 0.15 m^2 polyethersulfone hollow-fiber membrane, solicited from Membrana GmbH, was utilized in place of the sediment filters. This membrane was intend to be used throughout the study, however, due to fouling and issues with the housing of the membrane, the Membrana membrane was unusable after day 196, and sediment filters were used for the remainder of the study. Sediment filters were able to maintain an average effluent flow rate of 0.4 L/d compared to 0.6 L/d with the working Membrana membrane. As a result, SRT in the methane phase was reduced from the originally intended 75 days to 30 days with an average sludge waste rate of 0.35 L/d. To overcome fouling, sediment filters were replaced approximately every 50-100 days.

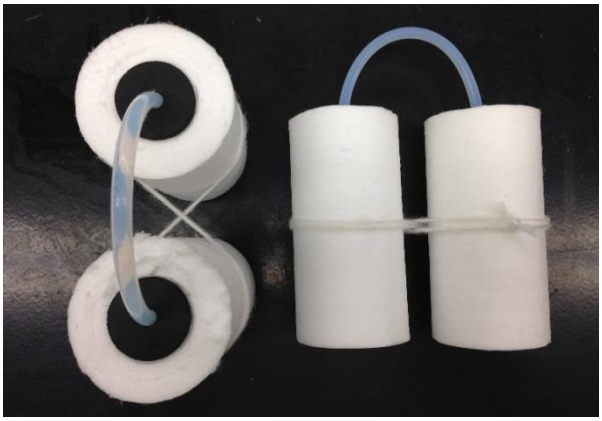


Figure 3.3. Modified sediment filters prior to application in the AnMBR system.

Three periods of operation were investigated in this study. Table 3.2 provides a summary of the operating conditions for each of the three periods of operation. During the first period of operation, referred to as *Bioaugmentation 1*, daily bioaugmentation in the acid-phase consisted of addition of a 50:50 mixture (VS basis) of anaerobic sludge recycled from the methane-phase

reactor and the dry proprietary bioculture blend. This mixture was added to the acid-phase at a dosage of 3.9% of daily influent VS. During the second period of operation, referred to as *No Bioaugmentation*, all bioaugmentation in the acid-phase was stopped. During the third and final period of operation, referred to as *Bioaugmentation 2*, a second bioaugmentation strategy was applied to the acid-phase. This time, only the dry proprietary bioculture blend was used for bioaugmentation with no recycling of anaerobic sludge. In this case, the proprietary bioculture mixture made up the entire dosage of 3.9% of daily influent VS.

Table 3.2. Operating conditions (after start-up) in the AnMBR system.

Parameter	Unit	Bioaugmentation 1		No Bioaugmentation		Bioaugmentation 2	
		Acid-phase	Methane-phase	Acid-phase	Methane-phase	Acid-phase*	Methane-phase
Period of Operation	Days	126-226		227-374		375-653	
Total OLR	(g COD/L d^{-1})	2.44 ±0.9		1.86 ±0.3		1.76 ±0.5	
HRT	Days	2	16	2	16	2	16
SRT	Days	2	75-30	2	30	2	30
Temperature	°C	37 ±3	37 ±1	37 ±3	37 ±1	37 ±3	37±1
pH		4.7 ±0.2	7.5 ±0.1	5.4 ±0.7	7.5 ±0.1	5.3 ±0.5	7.5±0.1
Bioaugmentation		Recycled sludge + Proprietary Mix 3.9% of feed VS		—		Proprietary Mix 3.9% of feed VS	

**The acid-phase was operated under Bioaugmentation 2 conditions for an additional 300 days beyond this study.*

Analytical Methods

Over the course of this study, regular analysis of chemical oxygen demand, total and volatile solids, and sulfide analysis were carried out according the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Sulfide analysis was carried out using the Methylene Blue Method using a HACH visual test kit. Volatile fatty acid analysis was conducted by the Metabolmics Center at the University of Illinois at Urbana-Champaign using GCMS. Biogas samples were collected regularly from the methane-phase of the AnMBR system via a syringe and 7 mL Vacutainer sample vials (BD Vacutainer, 8020128). Biogas quality was measured by gas chromatography (Varian, Model CP-3800), equipped with an Alltech Hayesep D 100/120 column and a thermal conductivity detector (TCD). The carrier gas was helium at a flow rate of 30 mL/min. Temperature of both the injector and detector was 120˚C.

Statistical Methods

The majority of analytical values are reported as means with standard deviation. Two-tailed Student's T-tests with unequal variance were conducted for comparison of statistical difference among data sets with $n < 30$ using a 95% confidence interval ($p \leq 0.05$). For data sets with $n > 30$, a two-sample Z-test was conducted, again using a 95% confidence interval ($p \leq 0.05$). Both statistical tests were carried out in Microsoft Office Excel 2013.

Microbial Community Analysis

Samples of the propriety bioculture, influent, acid-phase, and methane-phase were collected throughout the study for microbial community analysis. 25 ml samples from the acid- and methane-phase were collected during the latter part of each operating period on the following days of operation: *Bioaugmentation 1* - Day 169, 215, and 221; *No Bioaugmentation* - Day 349, 460, 465; *Bioaugmentation 2* acid-phase – 485, 541, 641, 646, 727, 886, and 939; *Bioaugmentation 2* methane-phase - Day 541, 641, and 646. Influent samples were collected on days 102, 169, 349, 467, 543, and 718. DNA extraction from the samples was carried out using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, LLC) using the manufacturer's instructions. The extracted DNA was stored at -20°C until submitted for sequencing. Sequencing of the extracted DNA was carried out by the University of Illinois Keck Center using Illumina Miseq sequencing combined with Fluidigm sample preparation. Primer pair V4-515F - V4-806R was used to amplify the V4 region of the 16S rRNA gene of bacteria. Mothur version 1.35.0 (Schoss et al. 2009) was used to assemble the forward and reverse sequences using the standard operating procedure for Miseq data (Kozich et al., 2013 accessed January 2015). Sequences that appeared 3 times or less in the entire data set were removed. Alignment and taxonomic classification was done using the Silva Bacterial reference database, release 102, as provided by Mothur (Schloss et al., 2009). Using the software Primer-E Version 7 (Quest Research Limited, Auckland, New Zealand), the aligned sequence data was used to generate multidimensional scaling (MDS) plots, using Bray-Curtis dissimilarity index to visualize the similarity among microbial communities of the three different operating conditions.

Results and Discussion

Acid-phase sCOD and VFA Production With and Without Bioaugmentation

The generation of sCOD in the acid-phase was used to evaluate the impact of bioaugmentation on acid-phase hydrolysis. Figure 3.4a shows sCOD concentrations in the influent and acid-phase during each period of operation. During the first period of bioaugmentation, *Bioaugmentation 1*, average acid-phase sCOD concentrations were significantly higher compared to operation without bioaugmentation ($p=3.88x10^{-18}$). This was 48.8% greater than that during *No Bioaugmentation* (10,532 compared to 7,081 mg/L). Compared to average influent sCOD concentrations, which were similar during *Bioaugmentation 1* and *No Bioaugmentation* (p=0.860), these values corresponded to a percent increase of 108.4% and 37.8% respectively. Therefore, bioaugmentation with recycled sludge and the dry proprietary bioculture blend had an obvious positive impact on substrate hydrolysis compared to operation without bioaugmentation.

In the case of *Bioaugmentation 2*, average acid-phase sCOD concentration was not significantly different than that during *No Bioaugmentation* (6,943 mg/L compared to 7,080 mg/L, p=0.557). This corresponded to a 14.4% increase from average influent sCOD. This percent increase was less than that during both *Bioaugmentation 1* and *No Bioaugmentation*. The reason for decreased average sCOD generation during *Bioaugmentation 2*, this likely due to the fact that the influent during *Bioaugmentation 2* was initially more solubilized than during previous operating periods. During *Bioaugmentation 2*, between days 560 to 653, the average percentage of sCOD in the influent was 21%, compared to 12% and 16% during *Bioaugmentation 1* and *No Bioaugmentation*, respectively. Given the higher fraction of influent sCOD and considering that most hydrolytic bacteria are also capable of fermentation, it is likely that these bacteria would have converted already solubilized substrates to volatile fatty acids and other fermentation intermediates prior to hydrolyzing un-solubilized complex particulate organic matter (Gerardi, 2006). Thus, acid-phase hydrolysis would have been less of a limiting factor during this period of operation. In order to better evaluate the potential benefit of bioaugmentation with the proprietary bioculture alone on substrate hydrolysis, operation of the acid-phase reactor under *Bioaugmentation 2* conditions continued for an additional 300 days during which the average percentage of sCOD in the influent was 17%, a value more comparable

to that during *No Bioaugmentation*. During this period, average acid-phase sCOD concentration was 8,183 mg/L which was significantly higher compared to that during *No Bioaugmentation* (p=0.0001), however this still corresponded to a lower percent increase (25.7%) from influent sCOD concentrations compared to operation during *Bioaugmentation 1* and *No Bioaugmentation*. Overall, since the greatest increase in sCOD was observed during *Bioaugmentation 1*, substrate hydrolysis was likely benefited more by recycling of the anaerobic sludge compared to bioaugmentation with the proprietary bioculture. The beneficial impact of bioaugmentation with the proprietary bioculture on acetogenesis was more evident.

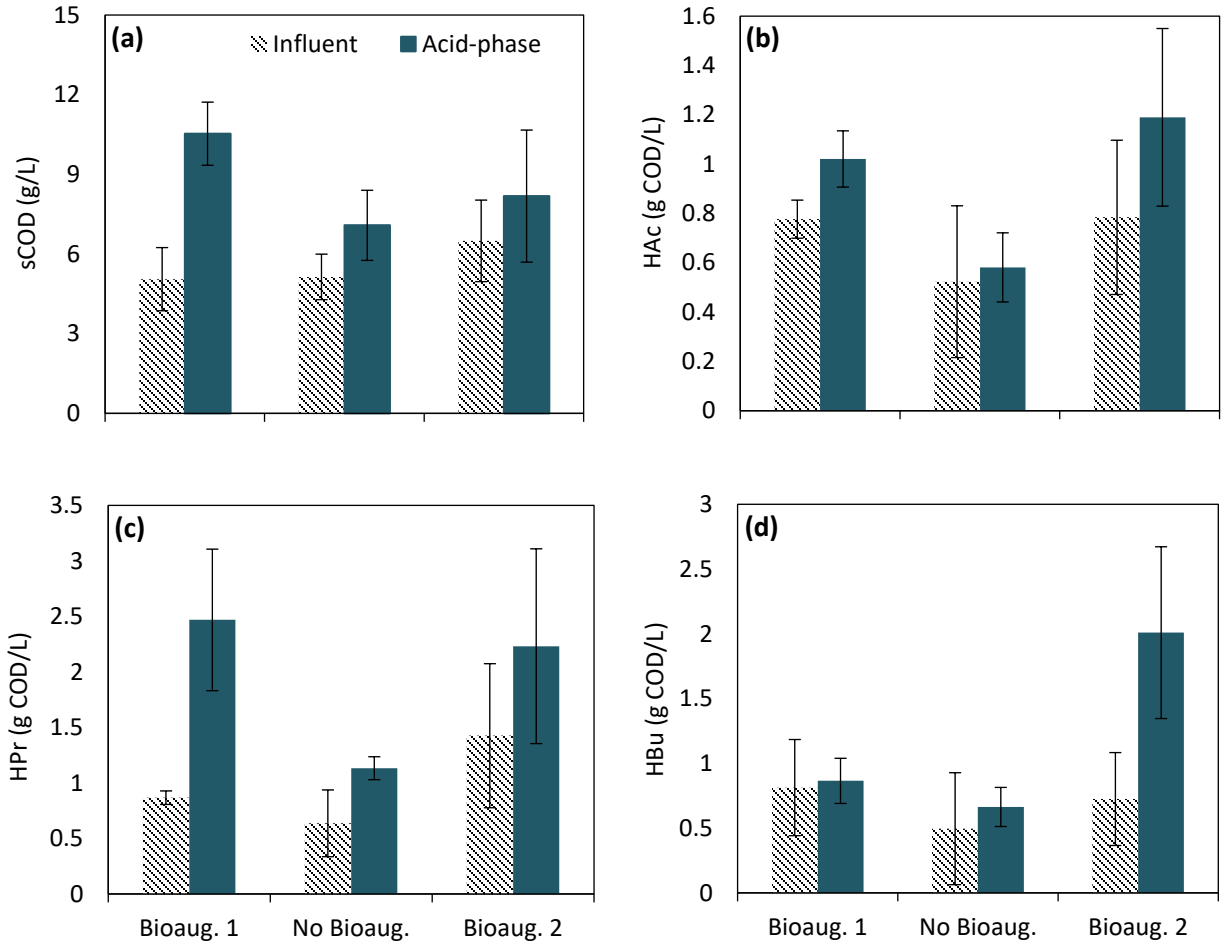


Figure 3.4. Average influent and acid-phase (a) soluble COD, (b) acetic acid, (c) propionic acid, and (d) butyric acid concentrations during operation with and without bioaugmentation. Statistical differences were determined using Student's T-test ($p<0.05$).

Both bioaugmentation strategies resulted in significantly greater VFA production in the acid-phase, and in particular, higher average acetic acid levels, compared to operation without bioaugmentation ($p=5.01x10^{-17}$ and $p=0.0004$). Figures 3.4b, c and d show acetic, propionic, and butyric acid concentrations, respectively, in the influent and acid-phase during each period of operation. Average acid-phase acetic acid concentrations increased from influent levels by 31 and 52% during *Bioaugmentation 1* and *2*, respectively, with average acid-phase concentrations of 1,021 and 1,190 mg COD/L respectively. In contrast, only an 11% increase in acetic acid concentration was observed during operation without bioaugmentation with an average acid-phase acetic acid concentration of 581 mg COD/L. The lower rate of acetogenesis in the non-bioaugmented condition could have been due to limited hydrolysis, and thus a lack of available soluble intermediates for conversion to acetate, or possibly due to a lower abundance of acetogenic bacteria compared to the bioaugmented conditions.

Higher levels of propionic and butyric acid were also observed in bioaugmented conditions compared to non-bioaugmented conditions which is consistent with the speculation that hydrolysis was limited during operation without bioaugmentation. Average propionic acid levels in *Bioaugmentation 1* and *2* were 2,468 and 2,231 compared to 1,132 mg COD/L without bioaugmentation ($p=1.3x10^{-8}$ and $p=0.001$). This corresponded to an increase of 184.8% and 56.6% during *Bioaugmentation 1* and *2*, respectively, compared to 105% without bioaugmentation. The significantly higher initial concentration of propionic acid in *Bioaugmentation 2*, compared to *Bioaugmentation 1* and *No Bioaugmentation* (1,425 mg COD/L compared to 866.7 and 636.1 mg COD/L, respectively) reflected the higher degree of initial solubility in the influent during *Bioaugmentation 2* compared to the previous periods. Average butyric acid levels in *Bioaugmentation 1* and *2* were 865 and 2,009 mg COD/L, respectively, compared to 663 mg COD/L without bioaugmentation. In this case average butyric acid concentration increased by 6.4% and 177% during *Bioaugmentation 1* and *Bioaugmentation 2* respectively, compared to 33.9% during *No Bioaugmentation*.

Bioaugmentation with the proprietary bioculture alone was investigated due to that fact that during *Bioaugmentation 1*, elevated sulfide concentrations, up to 440 mg/L in the methane-phase, were observed. These levels were 10 times higher than sulfide concentrations during operation without bioaugmentation. Figure 3.5 shows sulfide concentrations in the different phases of the continuous AnMBR system over time. Sulfide levels decreased shortly after

stopping *Bioaugmentation 1*, which raised the question of whether recycling of the anaerobic sludge could have contributed to an accumulation of sulfate reducing bacteria (SRB) during *Bioaugmentation 1*. This motivated investigation of the second period of bioaugmentation, *Bioaugmentation 2*, this time eliminating the recycled anaerobic sludge component. During *Bioaugmentation 2*, sulfide concentrations remained below 40 mg/L, which could suggest that recycling of the anaerobic sludge benefited SRB accumulation. However, it is more likely that the influent during *Bioaugmentation 1* had higher initial sulfate levels compared to later operating periods.

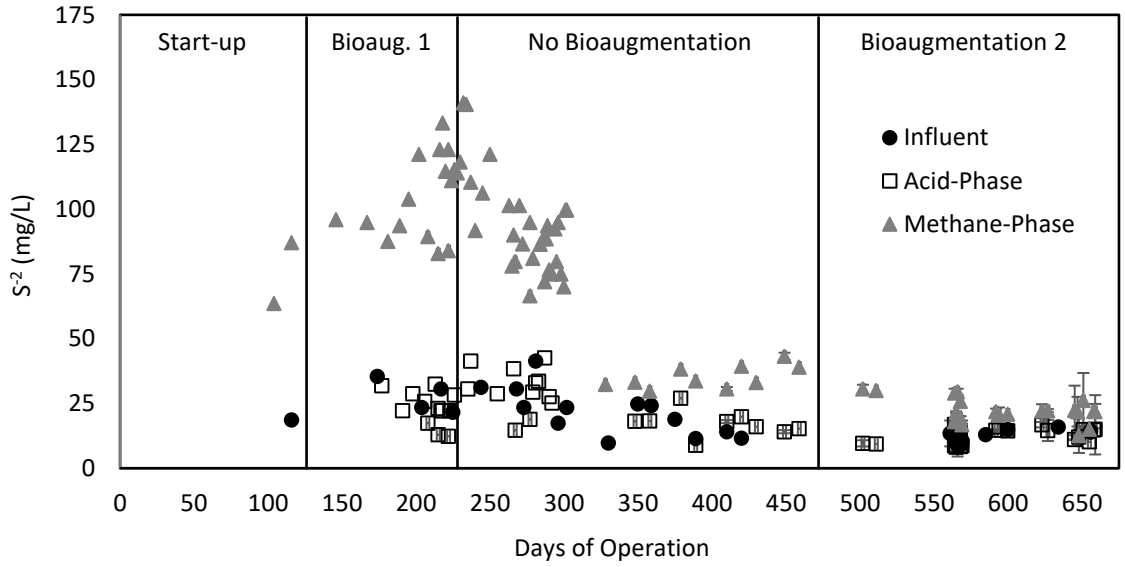


Figure 3.5. Measured sulfide concentrations in the influent, acid-phase, and methane-phase of the AnMBR system during each period of operation with and without bioaugmentation.

Methane Production and COD Removal With and Without Bioaugmentation

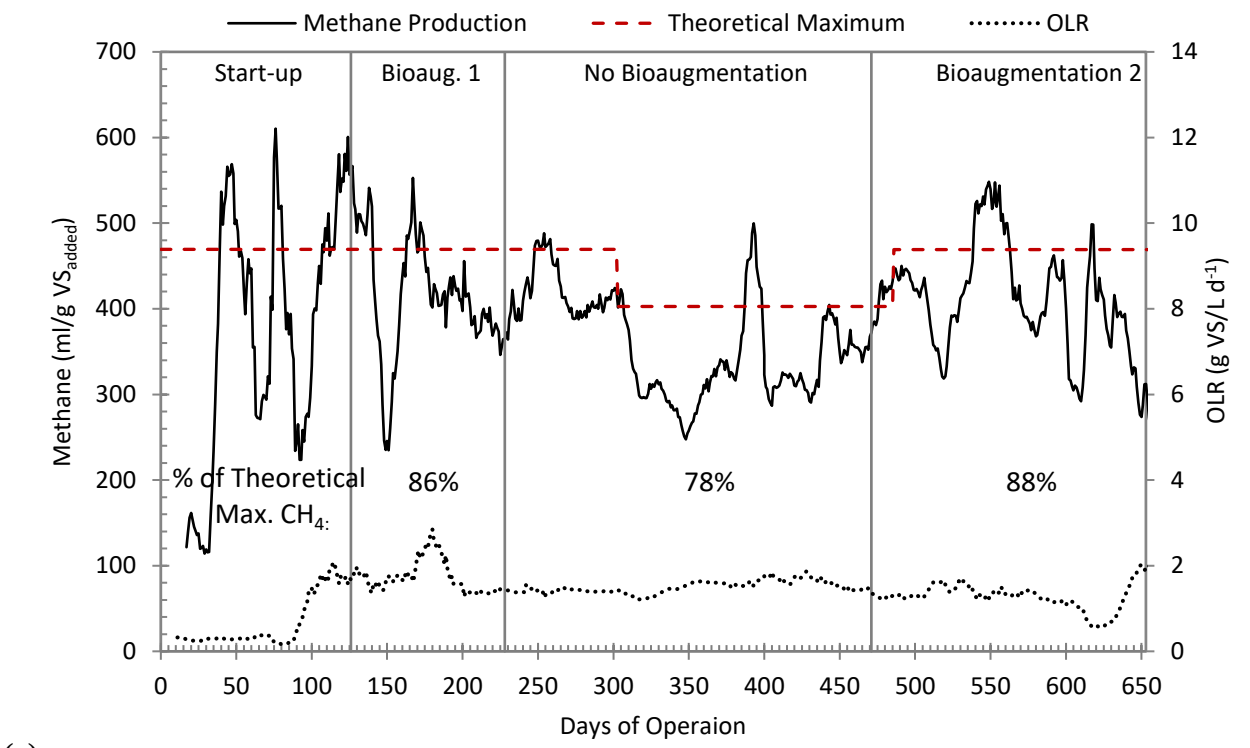
The benefit of bioaugmentation on substrate hydrolysis and acetogenesis were reflected in subsequent increases in methane production. Figure 3.6 shows average methane production per gram of VS_{added} in the AnMBR system over time. Average methane production during normal operation without bioaugmentation was 356 ml/g VS_{added}, 78% of theoretical maximum. This was similar to methane production from AnMBR treatment of sewage sludge seen in previous studies. Ghyoot and Verstraete (2010) reported methane production values between 142-322 ml/g VS_{fed} in a membrane-assisted sludge digester treating raw primary sludge under

similar operating conditions: 35°C, OLR of 1.06 kg COD/m^3 d^{-1}, and 20 day HRT. Similarly, Meade et al. (2013) reported an average methane yield of 242 ml/g VS_{fed} in a mesophilic AnMBR treating sewage sludge. With bioaugmentation, average methane production was significantly higher than that during operation without bioaugmentation ($p<1.51x10^{-13}$) increasing to 400 and 414 ml/g VS_{added} during *Bioaugmentation 1* and *2*, respectively (86% and 88% of theoretical maximum methane production). The average percent of methane in the biogas was 68, 71, and 76% during *Bioaugmentation 1*, *No Bioaugmentation*, and *Bioaugmentation 2*, respectively. The lower percentage of methane observed in *Bioaugmentation 1* could be a result of the previously discussed increased in sulfide reduction that was observed during this period of operation. Diversion of acetate and hydrogen substrates from methanogenesis to the more energetically favorable route out of sulfate reduction would have reduced overall methane production and increased the proportion of hydrogen sulfide in the biogas.

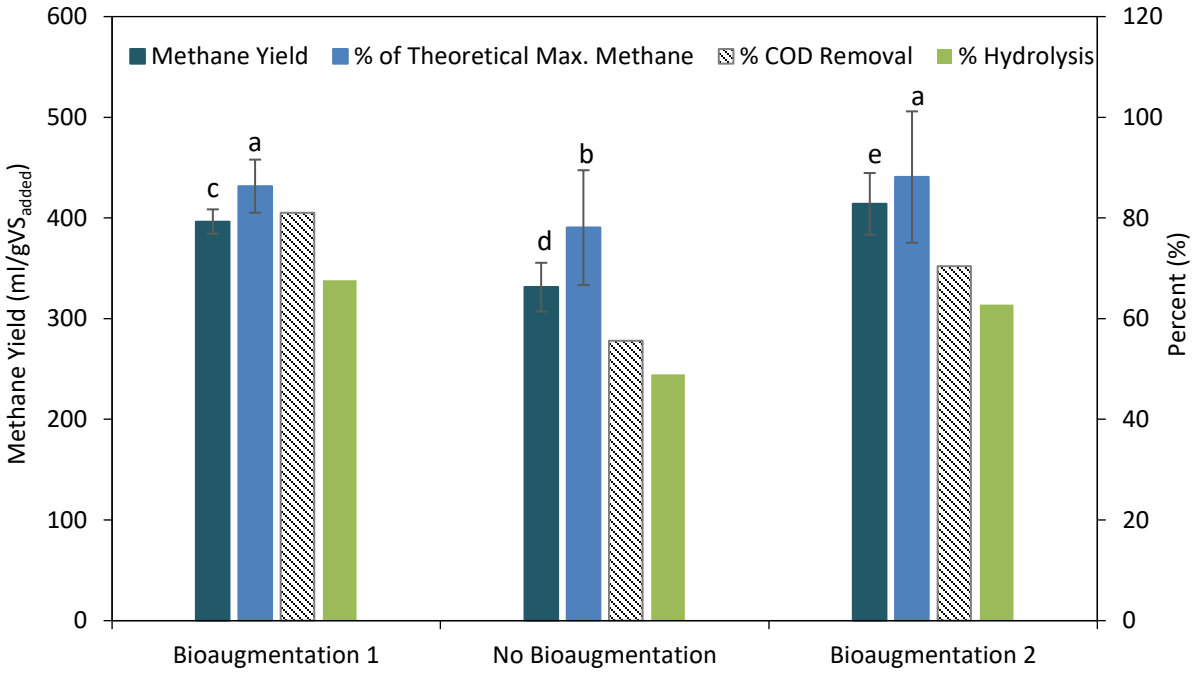
Corresponding with increased methane production, greater COD removal was also achieved during operation with bioaugmentation compared to operation without. Table 3.3 summarizes average COD mass flows during each period of operation. During operation without bioaugmentation, 52% COD removal was achieved. This is similar to COD removals achieved in previous studies. In the membrane-assisted digester of Ghyoot and Verstraete (2010), a maximum COD removal of 54% was achieved, while Pierkiel and Lanting (2005), observed a 59% VS reduction in a mesophilic membrane-coupled anaerobic digester treating mixed primary and secondary sludge. With bioaugmentation, average COD removal increased by 25-55%, with average overall COD removals of 81 and 66% during *Bioaugmentation 1* and *2* respectively.

Table 3.3. Organics mass balance, specific methane production, and percent of methane in the biogas during for each period of operation in the two-phase AnMBR system.

(g/d)	$COD_{Influent}$	$COD_{Permeate}$	COD_{Sludge}	$COD_{Removed}$	$COD_{Methane}$	CH_4 (m^3/kg COD)
Bioaugmentation 1	32.9 ± 4.0	0.18 ± 0.02	6.1 ± 0.83	26.7	24.6 ± 1.5	0.26
No Bioaugmentation	25.0 ± 7.6	0.09 ± 0.03	12.0 ± 4.0	13.2	14.8 ± 2.0	0.21
Bioaugmentation 2	23.8 ± 7.0	0.07 ± 0.04	8.1 ± 3.3	15.6	16.9 ± 2.5	0.25
% of Influent	$COD_{Influent}$	$COD_{Permeate}$	COD_{Waste}	$COD_{Removed}$	$COD_{Methane}$	CH_4 % of Biogas
Bioaugmentation 1	100	0.53	18.5	81.0	74.7	68.0 ± 0.03
No Bioaugmentation	100	0.37	47.3	52.4	58.9	71.2 ± 0.03
Bioaugmentation 2	100	0.28	34.2	65.5	71.2	76.4 ± 0.03



(a)



(b)

Figure 3.6. (a) Methane production in the AnMBR system over time, and (b) average methane yield, percent of theoretical maximum methane production, overall COD removal, and percent of hydrolysis in the methane-phase for each operating period. Significant differences were determined using two-mean Z-test ($p<0.05$).

The higher COD removal achieved with bioaugmentation reflects the greater extent of hydrolysis that occurred in these conditions compared to operation without bioaugmentation. Thus, in the case of *Bioaugmentation 2*, where a lower percent increase in sCOD was observed in the acid-phase, a higher overall rate of hydrolysis was achieved during this operating period compared to *No Bioaugmentation*. According to Miron et al. (2000), approximately 20 and 60% of particulate biopolymers are hydrolyzed under acidogenic and methanogenic conditions, respectively. The percent of hydrolysis in the acid and methane-phase of each of the three conditions were calculated using the following relation from Halalsheh et al. (2005):

$$Percent\ of\ Hydrolysis = \frac{COD_{CH_4} + (sCOD_{out} - sCOD_{in})}{(tCOD_{in} - sCOD_{in})}\ x\ 100$$

where $sCOD_{out}$ is the soluble COD of the effluent, $sCOD_{in}$ is the soluble COD of the influent, and $tCOD_{in}$ is the total COD in the influent.

In calculating the percent of hydrolysis in the acid-phase, methane production was assumed to be zero given the short SRT. The resulting values for average percent of hydrolysis in the acid-phase were 14.1, 7.2, and 3% during *Bioaugmentation 1*, *No Bioaugmentation*, and *Bioaugmentation 2*, respectively, while average percent of hydrolysis in the methane-phase was 67.6, 48.9, 62.8%, respectively. These results indicated that although the percent of hydrolysis in the acid-phase was not enhanced in the case of *Bioaugmentation 2*, percent of hydrolysis in the methane-phase was, and thus, the overall percent of hydrolysis, was higher during both periods of operation with bioaugmentation compared to operation without.

In summary, performance measures of the two-phase AnMBR process investigated in this study indicated that bioaugmentation successfully increased rates of hydrolysis and acetogenesis compared to operation without bioaugmentation, leading to increased methane production and solids reduction.

Similarity/Dissimilarity of Bioaugmented versus Non-bioaugmented Microbial Communities

Seeing improvements in system performance as a result of bioaugmentation, microbial community analysis was conducted to relate the observed improvements with changes in microbial community composition. Approximately 3500 bacterial OTUs were identified among all samples analyzed in this study. These OTUs were used to generate multidimensional scaling plots (MDS) (Figures 3.7 and 3.8), using Bray-Curtis dissimilarity index to evaluate the

similarity/dissimilarity among microbial community samples collected from the acid- and methane-phases during the three different operating periods.

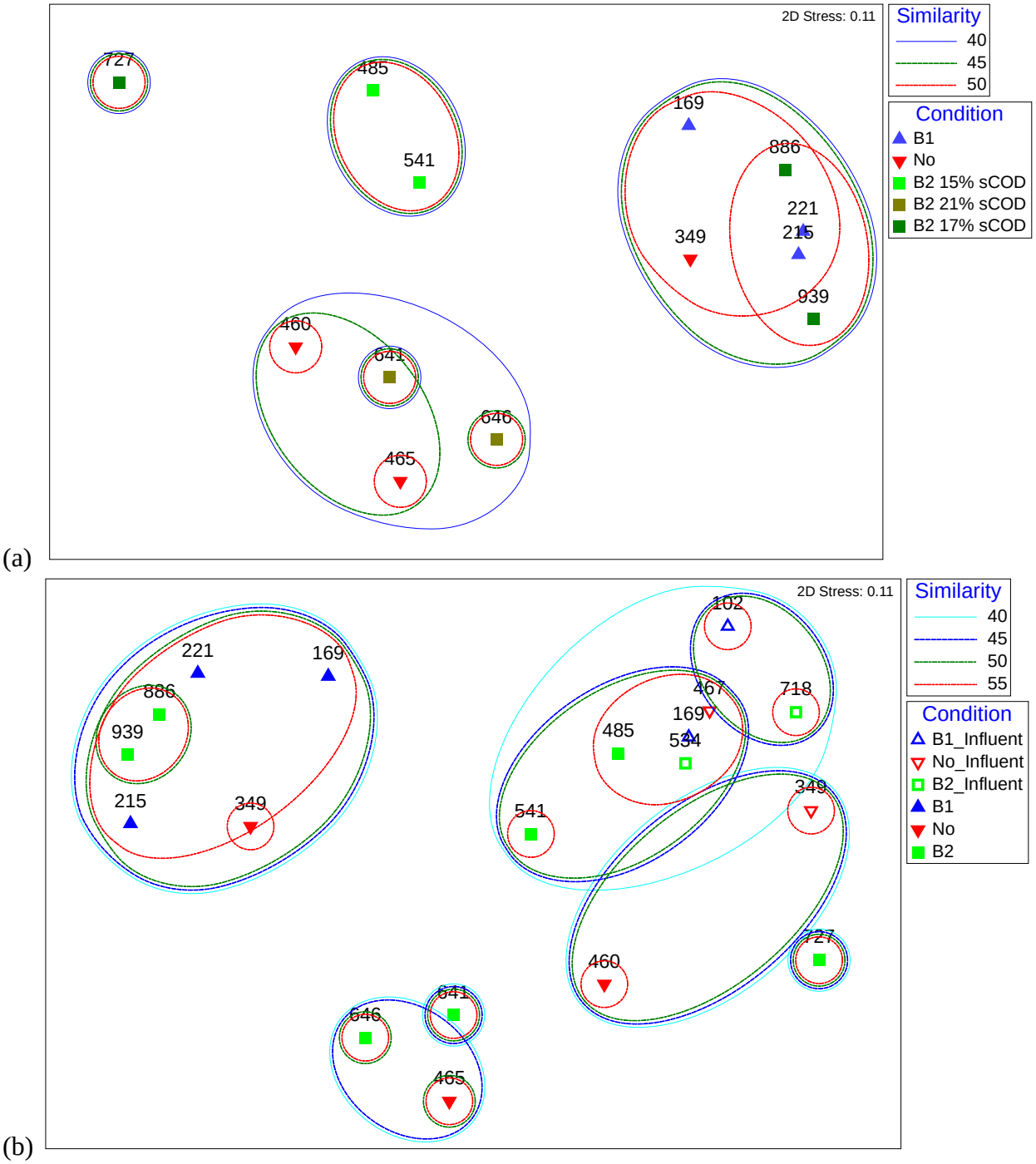


Figure 3.7. Bray-Curtis Similarity Index MDS plots of Bioaugmented and Non-bioaugmented bacterial communities in the (a) acid-phase only and (b) in relation to influent samples. Sample numbers refer to the day when they were collected. Influent sCOD percentage during *Bioaugmentation 2* operation is also indicated in Figure 3.7a.

Looking first at acid-phase bacterial communities in Figure 3.7a, it was found that for the most part communities grouped according to operating condition. All *Bioaugmentation 1* samples grouped together with 59% similarity. An interesting trend was observed in the *No Bioaugmentation* condition. In this case, the later two out of three samples grouped more similarly to each other (49.3% similarity) than to the first sample, which grouped more similarly to *Bioaugmentation 1* samples (53.8% similarity). It is suspected that this trend of later samples grouping more closely to each other than the first sample may reflect the transition time that it took for the bacterial community to shift. During *Bioaugmentation 2,* as similar shifting over time was observed among the different samples collected. The first samples collected during *Bioaugmentation 2* group together with 51.0% similarity and separately from the other operating conditions. The influent during this time was of the same batch fed during the *No Bioaugmentation* operating period, with an average sCOD percentage of 15%. However, the following two samples collected during *Bioaugmentation 2* shifted away from the initial two samples, with one grouping with 40.3% similarly to the *No Bioaugmentation* samples. These samples had been collected during the time when the influent was quite different than previous operating periods, with a relatively high average sCOD percentage of 21%. In this case, because the influent was already quite solubilized, proliferation of hydrolytic microorganisms potentially coming from the bioculture may have been limited since there was less of a need for increased hydrolysis. When sCOD in the influent reduced to a level similar to the previous period of operation (17%), another shift in the microbial community was observed, back towards the initially collected *Bioaugmentation 2* samples. Finally, the last two *Bioaugmentation 2* samples were observed to group similarly to each other (60.0% similarity) and to the *Bioaugmentation 1* samples (46.1% similarity). This suggested that bioaugmentation had a relatively consistent impact on the microbial community during the two different periods of operation with bioaugmentation. Some of the dissimilarity between *Bioaugmentation 1* and *Bioaugmentation 2* samples is likely related to the recycling of anaerobic sludge that occurred during *Bioaugmentation 1* but not during *Bioaugmentation 2*. In addition, the influent, which was more variable in the case of *Bioaugmentation 2*, would have also contributed to some of the dissimilarly observed among the Bioaugmentation 1 and 2 communities. However, as shown in Figure 3.7b, which visualized the Bray Curtis similarity among both influent and acid-phase samples, variation of the influent alone could not explain the changes observed among microbial

communities since there was little grouping of acid-phase samples with their corresponding influent samples. Overall, it appeared that bioaugmentation did have an impact on acid-phase microbial community structure, with bioaugmented communities, under low influent sCOD conditions, ultimately grouping more similarly to each other than to non-bioaugmented communities.

Compared to acid-phase communities, less similarity among methane-phase bacterial communities in the two bioaugmented conditions was observed, as shown in Figure 3.8. As a group, *Bioaugmentation 1* samples grouped most similarly, with 56.3% similarity. The earliest sample from *No Bioaugmentation* again grouped more similarly to *Bioaugmentation 1* samples, while the later two samples grouped more similarly to *Bioaugmentation 2* samples, 64%. Similarity among *Bioaugmentation 2* samples was relatively low, 27.5%, with the majority of samples having greater similarity to non-bioaugmented samples than *Bioaugmentation 1* samples. The stronger grouping of *Bioaugmentation 1* samples relative to the other two conditions could be related to the recycling of anaerobic sludge in this condition, which may have reinforced the methane-phase community. Overall, examination of Bray Curtis Similarity suggested that bioaugmentation had the greatest impact on acid-phase bacterial communities with bioaugmented samples grouping more similarly to each other than to non-bioaugmented.

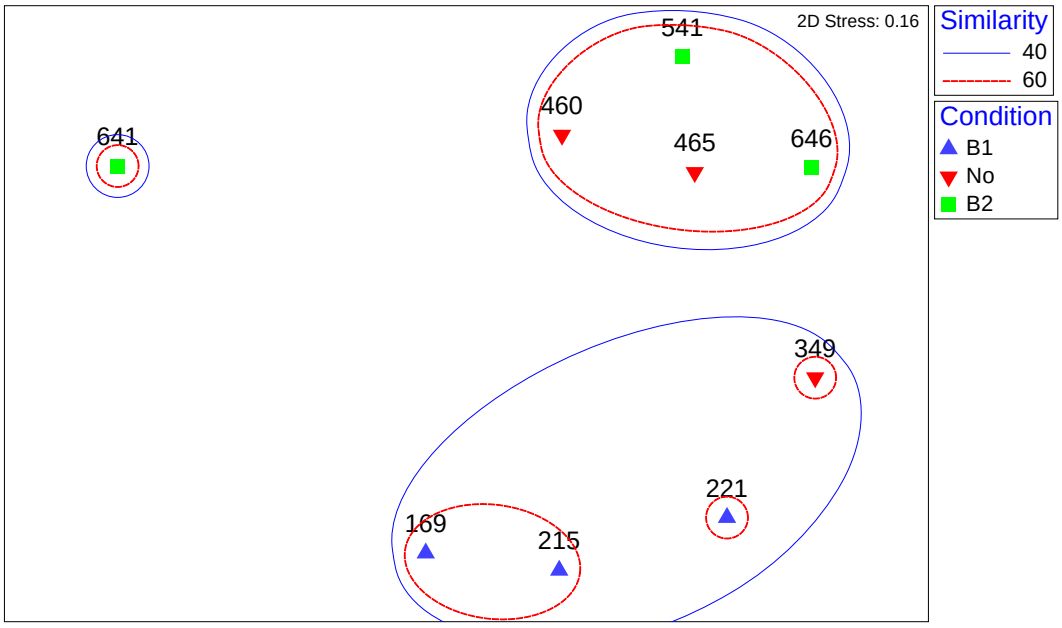


Figure 3.8. Bray-Curtis Similarity Index MDS plot of Bioaugmented and Non-bioaugmented bacterial communities in the methane-phase of the two-phase AnMBR process. Sample numbers refer to the day when they were collected.

Identification of Microbial Players in Bioaugmented Communities

Seeing some similarity among bioaugmented communities, the distribution of OTUs coming from the proprietary bioculture within bioaugmented versus non-bioaugmented communities was investigated to further elucidate the impact of bioaugmentation on microbial community structure. 81 bacterial OTUs were identified in the bioculture. These OTUs represented 6 different phyla as shown in Figure 3.9a. The majority of bioculture OTUs (64%) belonged to the phylum Firmicutes, which is known to contain numerous hydrolytic and fermenting bacteria such as species from the genera *Streptococcus*, *Clostridium*, and *Bacillus* (Shah et al., 2014). Of the total 81 OTUs, 34 were also found in the influent, however, the majority (30 out of 34) of these OTUs had a relative abundance 2-24,000 times greater in the bioculture compared to the influent. The four OTUs that were present in a higher proportion in the influent were not considered as having come from the bioculture in further analyses. Recycled sludge OTUs were defined as those OTUs that were identified in the methane-phase during *Bioaugmentation 1* and that were not identified in the bioculture or influent. 182 OTUs were identified in the recycled sludge. The majority of these OTUs (42%) were unclassified Bacteria. The majority of classified OTUs were associated with the phyla Bacteroidetes (23%) and Firmicutes (15%). The distribution of recycled sludge OTUs by phylum is shown in Figure 3.9b.

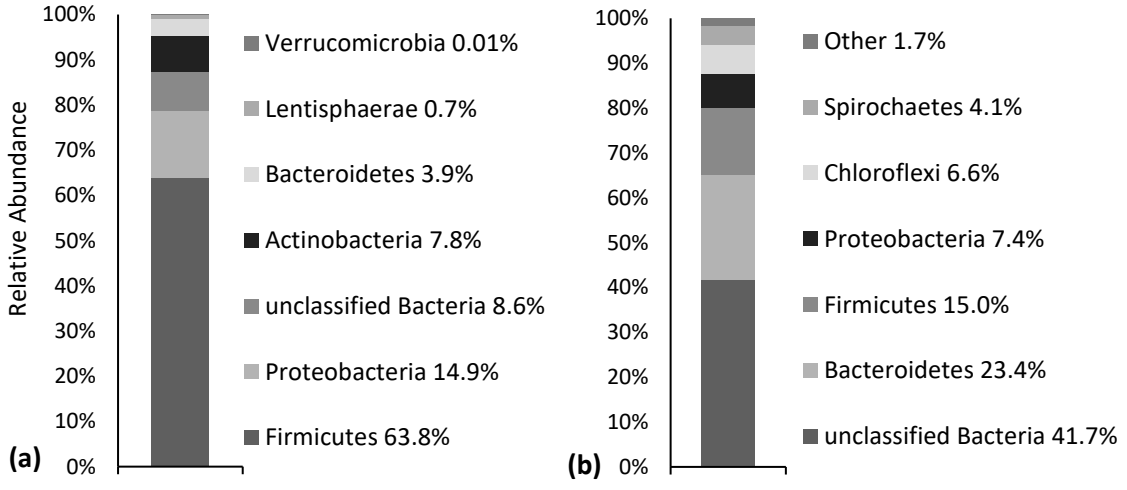


Figure 3.9. Relative abundance of all OTUs identified within the (a) propriety bioculture and (b) recycled anaerobic sludge grouped according to phylum level classification.

Figure 3.10a and b shows the average relative abundance of bioculture and recycled sludge OTUs, respectively, that were present within bioaugmented and non-bioaugmented samples. Results indicated that both bioaugmented conditions had a higher relative abundance of bioculture OTUs in the acid-phase compared to the non-bioaugmented condition. Bioculture OTUs represented 1.92% and 1.86% of total bacterial relative abundance in the acid-phase of *Bioaugmented 1* and *2* conditions, respectively, compared to only 1.1% in the non-bioaugmented condition. In the methane-phase, a higher relative abundance of bioculture OTUs was observed in *Bioaugmentation 2* (1.7%) compared to *No Bioaugmentation* and *Bioaugmentation 1* (1.5 and 0.5%, respectively). Looking at recycled sludge OTUs, as expected, a higher relative abundance of recycled sludge OTUs was identified in the acid-phase during *Bioaugmentation 1* compared to the later two operating periods: 21.0% compared to 12.8 and 12.4%.

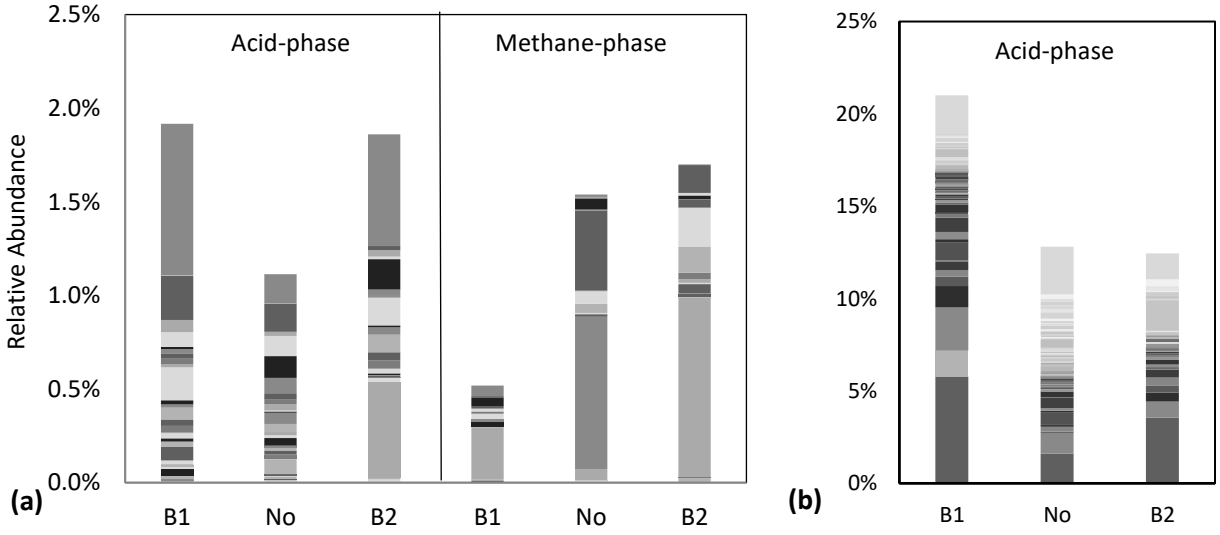


Figure 3.10. Average relative abundance of (a) bioculture OTUs identified within the acid- and methane-phase of bioaugmented (B1 and B2) and non-bioaugmented (No) communities, and (b) recycled sludge OTUs identified within acid-phase of bioaugmented and non-bioaugmented communities.

The relative abundance of OTUs that increased by more than 0.01% in the bioaugmented conditions compared to non-bioaugmented conditions are identified in Figure 3.11. In the figure, OTUs are identified at the genus level. OTUs that were unclassified at the genus level are labeled according to the next highest taxonomic level identified. In both bioaugmented conditions, the greatest increase was observed in an OTU of the genus *Acetobacter*. Most strains of *Acetobacter* bacteria are well known acetic acid producing bacteria (Raspor & Goramovic, 2008). The increase of this genus could be related to the increase in acid-phase acetic acid levels observed during operation with bioaugmentation. In the methane-phase, both bioaugmented conditions had a noticeable increase in the relative abundance of an OTU corresponding to the genus *Syntrophomonas*. Several species of the genus *Syntrophomonas* are known syntrophic acetogenic bacteria capable of degrading long-chain fatty acids as well as propionic and/or butyric acid to acetate (Cavaleiro et al., 2010; Shah et al., 2014). The increase of this genus could have benefited conversion of the higher soluble organics load and volatile fatty acids to acetate contributing to the increase in methane production observed during operation with bioaugmentation. While the higher relative abundance of these two genera may suggest that bioaugmentation did contribute to microbial community changes that benefited acetic acid production, it was surprising that the bioculture OTUs, particularly those associated with hydrolytic and fermentative bacteria, did not represent a greater relative abundance among the bioaugmented microbial communities. Recycling of the anaerobic sludge may have contributed to microbial community changes that led to the increase sCOD generation observed during *Bioaugmentation 1*. Figure 3.11c shows the relative abundance of OTUs identified in the recycled sludge that increased by more than 0.1% in the acid-phase during *Bioaugmentation 1* compared to the later two operating periods. In can be seen that several OTUs associated with the order Clostridiales and phylum Bacteroidetes, which are both known to contain several hydrolytic and acetogenic species, had increased relative abundance during *Bioaugmentation 1*.

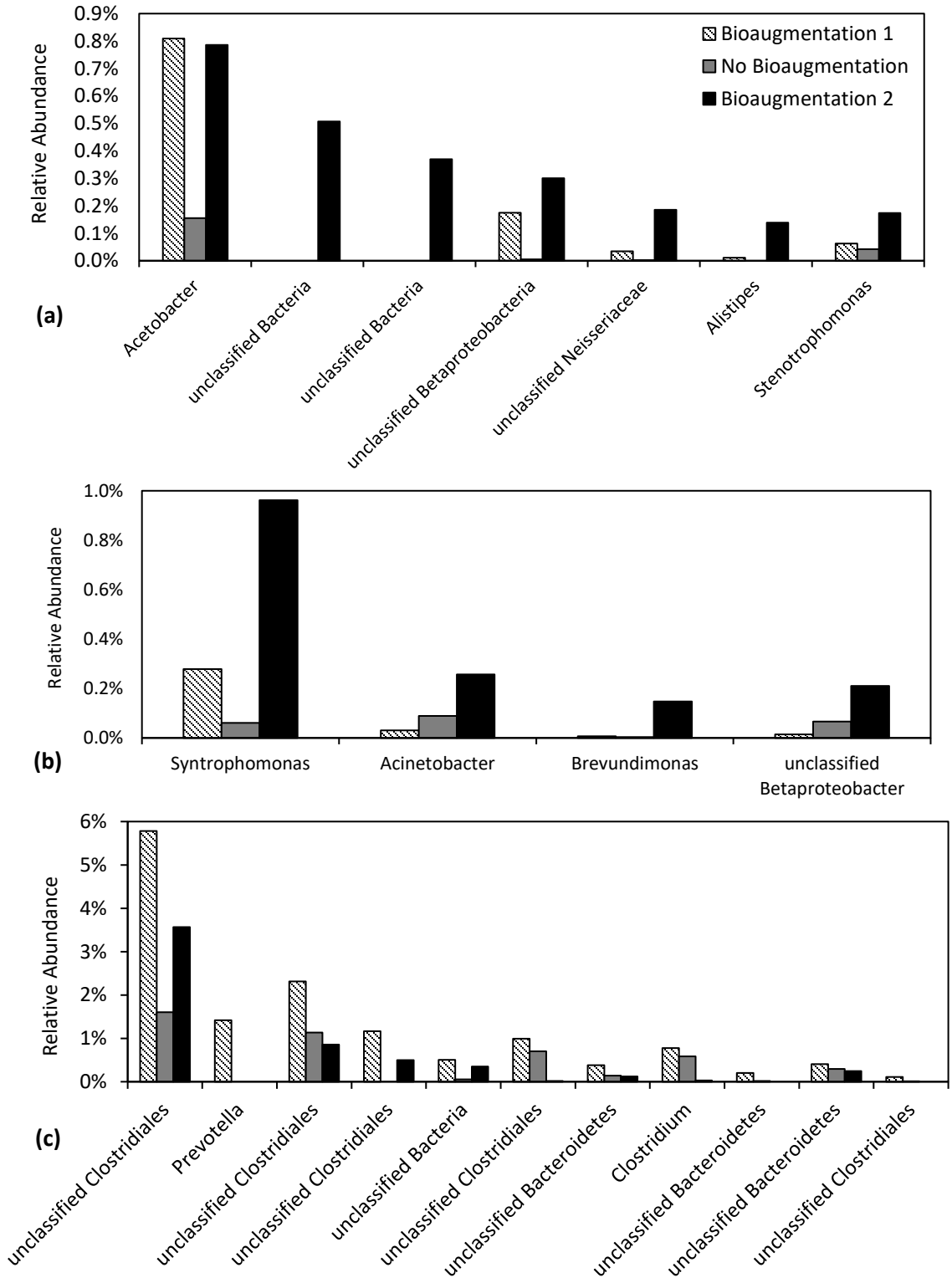


Figure 3.11. Relative abundance of bioaugmented OTUs that increased by more than 0.1% in bioaugmented conditions compared to non-bioaugmented conditions: (a) acid-phase bioculture OTUs, (b) methane-phase bioculture OTUs, and (c) acid-phase recycled sludge OTUs.

Overall, microbial community analysis revealed that changes in the microbial community did occur as a result of bioaugmentation, with notable increases in the relative abundance of OTUs associated with hydrolytic and acetic acid producing bacteria in bioaugmented conditions compared to non-bioaugmented conditions, although the relative abundance of bioaugmented microorganisms within bioaugmented communities was relatively low. Despite relatively low abundance, the reactor performance data showed that these relatively small changes can have a significant and noteworthy effect on anaerobic digestion. One limitation in this study was that anaerobic digestibility was already fairly high even without bioaugmentation. In a previous study, the authors showed that the effects of bioaugmentation can increase methane production by more than two times with more difficult to digest substrates (Martin-Ryals et al., 2015). Larger bioaugmentation benefits could also likely be fostered by more aggressive operating conditions such as shorter retention times, shock-loading, or lower operating temperatures. Therefore, while the results of this investigation were positive, and clearly show that bioaugmentation with hydrolytic and acetic acid producing bacteria can improve anaerobic process performance, future optimization of the bioculture composition should be explored in order to maximize the effectiveness of bioaugmentation. Finally, the dosage amount and frequency of bioaugmentation should be optimized. In this study, bioaugmentation was applied daily, however, this frequency could be potentially reduced in order to minimize the associated operating costs. Thus, future work should also investigate the longevity of the bioaugmented microorganisms and their effectiveness under a less frequent dosing regimen.

Conclusions

Bioaugmentation with hydrolytic and acetic acid producing bacteria is a promising strategy for improving solids reduction and overall efficiency of anaerobic treatment of sewage sludge. In this study, bioaugmentation using a proprietary bioculture as well as recycled anaerobic sludge in the acid-phase of a two-phase anaerobic membrane bioreactor (AnMBR) system treating primary sludge was investigated as a means for targeting and improving substrate hydrolysis and acetogenesis. Performance data indicated that bioaugmentation had a positive impact on substrate hydrolysis and acetic acid production achieving 25-38% greater average overall hydrolysis and increasing average acid-phase acetic acid levels by 31-52% compared to operation without bioaugmentation. These benefits led to subsequent increases in average methane production (10-13%) and greater average overall solids reduction (25-55%). Finally,

microbial community analysis using 16S Illumina MiSeq generated sequences confirmed increased relative abundance of bioaugmented microorganisms, including species related to acetic acid producing *Acetobacter*, and acetogenic *Syntrophomonas*, within bioaugmented communities compared to non-bioaugmented. Future optimization of the bioculture composition and dosing strategy could improve the effectiveness of bioaugmentation and reduce associated operating costs.

References

1. Angelidaki and B. Ahring. (2000). Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. *Water science and technology* 41(3): 189-194.
2. APHA. (2012). *Standard Methods for the Examination of Water and Wastewater* (22nd ed.). American Public Health Association, American Water Works Association, Water Environment Federation.
3. Cavaleiro, A.J. , Sousa, D.Z., Alves, M.M.. (2010). Methane production from oleate: Assessing the bioaugmentation potential of Syntrophomonas zehnderi. *Water Research*. 44(17): 4940-4947
4. Chang, S. (2014). Anaerobic Membrane Bioreactors (AnMBR) for Wastewater Treatment. *Advances in Chemical Engineering and Science*, *4*(1), 56–61.
5. Cirne, D. G., Lehtomäki, A., Björnsson, L., & Blackall, L. L. (2007). Hydrolysis and microbial community analyses in two-stage anaerobic digestion of energy crops. *Journal of Applied Microbiology*, *103*(3), 516–527.
6. Costa, J. C., S. G. Barbosa, M. M. Alves and D. Z. Sousa. (2012). Thermochemical pre- and biological co-treatments to improve hydrolysis and methane production from poultry litter. *Bioresource technology* 1111:41-147.
7. Diekert, G., & Wohlfarth, G. (1994). Metabolism of homoacetogens. In *Antonie van Leeuwenhoek* (pp. 66–209). Journal of Microbiology.
8. Eastman, J. & Ferguson, J. F. (1981). Solubilization of particulate organic carbon during the acid phase of anaerobic digestion. *Journal of the Water Pollution Control Federation*, *53*(3), 352–366.
9. Gerardi, M. H. (2006). *Wastewater Bacteria*. Wiley.
10. Ghyoot, W. R., & Verstraete, W. H. (1997). Coupling membrane filtration to anaerobic primary sludge digestion. *Environmental Technology*, *18*(6), 569–580.
11. Halalsheh, M., Koppes, J., den Elzen, J., Zeeman, G., Fayyad, M., & Lettinga, G. (2005). Effect of SRT and temperature on biological conversions and the related scum-forming potential. *Water Research*, *39*(12), 2475–2482.
12. Herrero, M., & Stuckey, D. C. (2015). Bioaugmentation and its application in wastewater treatment: A review. *Chemosphere*, *140*, 119–128.
13. Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology*, *79*(17), 5112–5120.
14. Martin-Ryals, A., Schideman, L., Li, P., Wilkinson, H., Wagner, R. (2015). Improving anaerobic digestion of a cellulosic waste via routine bioaugmentation with cellulolytic microorganisms. *Bioresource Technology*, 189:62-70
15. Miron, Y., Lier, J. B. V. A. N., & Lettinga, G. (2000). the Role of Sludge Retention Time in the Hydrolysis and Acidification of Lipids , Carbohydrates and Proteins During Digestion of Primary Sludge in Cstr Systems. Water Research, 34(5).
16. Mladenovska, Z., Y. Ishoy, A. Mandralioglu, P. Westermann and Ahring B. K. (2001). Bioaugmentation of a mesophilic biogas reactor by anaerobic xylanolytic- and cellulolytic bacteria. In *Proceedings of the 9th World Congress of Anaerobic Digestion*, 183. ed. Antwerp, Belgium
17. Mumme, J., B. Linke and R. Toelle. (2010). Novel upflow anaerobic solid-state (UASS) reactor. *Bioresource technology* 101(2): 592-599
18. Nielfa, A., Cano, R., & Fdz-Polanco, M. (2015). Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. *Biotechnology Reports*, *5*, 14–21.
19. Nielsen, H., Z. Mladenovska and B. Ahring. (2007). Bioaugmentation of a two-stage thermophilic (68 degrees C/55 degrees C) anaerobic digestion concept for improvement of the methane yield from cattle manure. *Biotechnology and bioengineering* 97(6): 1638-1643.

20. Pierkiel, A., & Lanting, J. (2005). Membrane-coupled anaerobic digestion of municipal sewage sludge. *Water Science & Technology*, *52*(1–2), 253–258.

21. Park, C., C. Lee, S. Kim, Y. Chen and H. Chase. (2005). Upgrading of anaerobic digestion by incorporating two different hydrolysis processes. *Journal of bioscience and bioengineering* 100(2): 164-167.

22. Prashanth, S., Kumar, P., Mehrotra, I. (2006). Anaerobic degradability: effect of particulate COD. Journal of Environmental Engineering. 132(4):488-496

23. Schloss, P. D. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541.

24. Shah, F.A, Mahmood, Q., Shah, M.M, Pervez, A., and Asad, S.A. (2014). Microbial Ecology of Anaerobic Digesters: The Key Players of Anaerobiosis. *The Scientific World Journal*, 183752, 21

25. Teo, C. W., & Wong, P. C. Y. (2014). Enzyme augmentation of an anaerobic membrane bioreactor treating sewage containing organic particulates. *Water Research*, *48*, 335–344.

26. Weiss, S., M. Tauber, W. Somitsch, R. Meincke and H. Muella. (2010). Enhancement of biogas production by addition of hemicellulolytic bacteria immobilized on activated zeolite. *Water research* 44(6): 1970-1980.

27. Yadvika, Santosh, Sreekrishnan, T.R., Kohli, S., Rana, V. (2004). Enhancement of biogas production from solid substrates using different techniques – a review. Bioresource Technology. 95:1-10

CHAPTER 4: UTILIZING ION-EXCHANGE RESIN TO IMPROVE RECOVERY FROM ORGANIC SHOCK-LOADING IN AN ANMBR TREATING SEWAGE SLUDGE

Abstract

The addition of ion-exchange resin in a two-phase continuous AnMBR system treating primary sludge at ambient temperature (20°C) was investigated as a means to improve reactor recovery after organic shock-loading. Four commercially available anion-exchange resins were evaluated for their ability to sorb soluble organics, specifically volatile fatty acids (VFA), from AnMBR effluent. The strong-base resin, Purolite TANEX was determined the best resin for deployment in the continuous AnMBR having achieved the greatest removal of soluble chemical oxygen demand (COD) (up to 36%) and acetic acid (up to 48%) in preliminary batch testing. Addition of 100 and 300 g/L TANEX resin in the AnMBR system improved effluent quality reducing effluent COD concentrations by 48 and 75% respectively under normal operating conditions (OLR: 2.2 g COD/L d^{-1}). After shock-loading with 16,000 mg COD/L acetic acid, reactor recovery in terms of methane production was 9-58% faster with the addition of TANEX than without, achieving full recovery around 23 and 50 days under controlled pH conditions (pH: 7.4). After shock-loading the system twice without the addition of TANEX, it was found that recovery without the addition of TANEX improved from 68 to 55 days, suggesting that acclimation of the microbial community also played a role in reactor recovery. Microbial community analysis using 16S Illumina MiSeq sequencing confirmed changes in the microbial community did occur as a result of shock-loading, with higher relative abundance of *Methanoscarcina* in the majority of post-shock-load microbial communities. The highest relative abundance of *Methanoscarcina* (51-58%) was seen during operating periods with the addition of TANEX resin, leading to the conclusion that addition of the TANEX resin benefited reactor recovery by reducing stress on the microbial community via sorption of excess acetic acid, allowing the community time to adjust and become better able to process the higher levels of acetic acid associated with the organic shock.

Introduction

Anaerobic membrane bioreactors (AnMBR) have become increasingly attractive as a means for treating municipal and industrial wastewaters, particularly those with high particulate solids content. The benefits of AnMBRs over conventional aerobic treatment include energy

recovery as methane, reduced energy input, reduced solids production, smaller footprint, and greater potential for nutrient and water recovery (Baloch and Akunna, 2002; Lin et at, 2013; Ozgun et al, 2013; Senturk, 2014; Stuckey, 2012). However, some drawbacks to anaerobic treatment include the slower growth rate of anaerobic microorganisms and greater susceptibility to upset or failure due to the delicate balance that must be maintained among the various microbial groups involved (Mitar et al, 1998; Ketheesan and Stuckey, 2015; Kim and Lee, 2015). Anaerobic treatment requires the coordinated metabolic interactions of several groups of microorganisms, namely hydrolytic, acidogenic, and acetogenic bacteria, as well as methanogenic archaea, to effectively transform organic waste from complex polymers to soluble monomer intermediates and then methane and carbon dioxide (Gujer and Zehnder, 1983; Ketheesan and Stuckey, 2015; Mosbaek et al, 2016). Accumulation of volatile fatty acid (VFA) intermediates due to shock or transient organic loading, temperature fluctuation, or changes in other operating parameters including pH, solid retention time (SRT), and/or hydraulic retention time (HRT), can inhibit methanogenesis leading to reactor upset and potential reactor failure (Ketheesan and Stuckey, 2015, Mosbaek, 2016;). Therefore, it is important to mitigate potential VFA accumulation and develop methods for achieving stable performance and effective reactor recovery in the case of fluctuating organic loads.

One potential method for mitigating the effect of organic shock-loading that leads to VFA accumulation is the addition of adsorbent or ion-exchange media. In this case, the adsorbent or ion-exchange resin acts as a temporary physio-chemical sink for excess dissolved VFA via interaction between the carboxylate group of the VFA and the active site of the adsorbent or ion-exchange resin's solid matrix (Rebecchi et al, 2016). This reduces the amount of soluble organics that escape from the AnMBR system and extends the time available for the microbial community to adjust and eventually convert the excess organic material into methane. Thus, organics removal, methane production, and effluent quality can all potentially be improved with the addition of adsorbent or ion-exchange materials. Previous literature has shown that the addition of adsorbents can be beneficial in mitigating organic shock-loading and improving COD removal, with the added benefit of improving membrane flux by providing physical scouring of the membrane surface (Akram and Stuckey, 2008; Hu and Stuckey, 2007; Park et el, 1999; Trzcinski, 2009; Yoo et al., 2012). One of the first studies of adsorbent addition was conducted by Park et al (1999) who found that addition of up to 5 g/L of powdered activated carbon (PAC)

enhanced both membrane flux and COD removal. Since then, addition of powered and granular activated carbon (GAC) in AnMBRs treating wastewater has been investigated by several researchers with fewer studies looking at the addition of ion-exchange resins (Akram, 2007; da Silva and Miranda, 2013). Ion-exchange resins can provide the same benefits of adsorbing soluble organics, and in fact, anion-exchange resins may provide better adsorption of VFAs than activated carbon. This is because activated carbon will adsorb mostly neutral compounds and a much smaller amount of positively and negatively charged organics compounds, whereas anion-exchange resins can selectively adsorb negatively charged organics compounds, i.e. VFAs. This was evident in a study by Hu and Stuckey (2007), where the addition of PAC was found to significantly increase overall COD removal, but only a small amount of VFA adsorption was observed. Similarly, da Silva and Miranda (2013) achieved greater adsorption of VFAs with a weak base ion-exchange resin than with activated carbon. Other benefits of ion-exchange resins relative to activated carbon include faster desorption kinetics due to more adsorption sites near the surface, which facilitates release of organics when microbes have depleted the bulk water concentrations, and because ion-exchange resins are flexible polymer structures concerns about membrane damage due to abrasion are reduced.

In this study, the application of an ion-exchange resin in a lab-scale continuous AnMBR system treating primary sludge under ambient temperature conditions was investigated. The objective was to determine whether the addition of an anion-exchange resin in the hydroxide form could improve process performance under ambient temperature conditions and improve reactor recovery after organic shock-loading events. It was hypothesized that exchange of hydroxide ion for VFA would help mitigate the pH change associated with organic-shock and that sorption of excess VFA via ion-exchange would reduce the loss of soluble organics in the effluent. Initially, four candidate ion-exchange materials were investigated and compared to GAC for their potential to adsorb soluble organics and VFA. The best of these materials was then deployed in the continuous AnMBR system and the effects in terms of effluent quality, methane production, and microbial community structure before and after organic shock-loading were investigated.

Materials and Methods

Primary Sludge Substrate

The substrate for this study was primary sludge collected from the Urbana-Champaign Sanitary District's (UCSD) Northeast Wastewater Treatment Plant. Primary sludge was collected at two different time points over the course of this study. The sludge was collected in 5 gallon plastic buckets and stored at 4°C until use. Table 4.1 provides characteristics of the two batches of primary sludge collected during this study. Elemental carbon, hydrogen, and nitrogen (CHN) analysis of the primary sludge was conducted by the University of Illinois at Urbana-Champaign (UIUC) Microanalysis Lab using a CHN analyzer (Exeter Analytical, Inc. CE-440). The percentage of oxygen was determined by subtracting the percentage of the other three elements and the ash content from 100. Theoretical maximum methane yield for the primary sludge substrate was then calculated based on elemental make-up and Boyle´s equation (Nielfa et al., 2014):

$$C_aH_bO_cN_d + \frac{(4a-b-2c+3d)}{4\,H_2O} = \frac{(4a+b-2c-3d)}{8\,CH_4} + \frac{(4a+b-2c-3d)}{8\,CO_2} dNH_3$$

Table 4.1. Characteristics of primary sludge substrate used over the course of this study.

Parameter	Units	Batch 1	Batch 2
Days of Use		0-132	132-375
Total Solids	g/L	24.2 ±3.3	24.0 ±3.2
Volatile Solids	g/L	19.5 ±3.4	18.1 ±0.4
Total COD	mg/L	36891 ±11684	39499 ±5944
Soluble COD	mg/L	6464 ±1300	7244 ±880
C:H:N:O *(by mass)*		43:6:3:31	42:6:4:29
Theoretical Maximum CH_4	mL/g VS	436.0 ±15.6	436.8 ±16.4
pH		5.43	5.58

Candidate Ion-exchange resins

Four candidate anion-exchange resins provided by Purolite®, were evaluated in terms of their ability to adsorb soluble COD, specifically negatively charged VFAs, in order to identify the best material for deployment into the continuous AnMBR system. Table 4.2 provides a description of the various candidate adsorbent materials that were investigated. Two different commercially available strong- and weak-base anion exchange resins were tested and compared

to GAC (Calgon Carbon Corporation, Pittsburgh, PA, USA). The two strong-based anion-exchange resins were obtained from the manufacturer in the chloride ion form, and utilized in this form for the initial batch screening tests. For application in the continuous AnMBR system, the TANEX resin was converted to the hydroxide ion form. Prior to all experimental testing, the adsorbed materials were rinsed with deionized water.

Table 4.2. Description of GAC and candidate ion-exchange resins.

Material	Type	Functional Group	Ionic Form	Size (mm)
FILTRASORB®400 GAC	adsorbent	activated carbon	na	1.2-1.6
Purolite® TANEX™	mixed strong base anion exchange resins	Quarternary Amine	Cl^- & OH^-	0.3-1.2
Purolite® A510	strong base anion exchange resin	Type II Quaternary Ammonium	Cl^-	0.3-1.2
Purolite® A845	weak base anion exchange resin	Tertiary Amine	OH^-	0.3-1.2
Purolite® A830	weak base anion exchange resin	Complex Amine	OH^-	0.3-1.2

Batch-test Screening of Candidate Ion-Exchange Materials

The candidate ion-exchange and GAC materials were initially loaded into 150 ml serum bottles containing filtered effluent collected during start-up of the AnMBR system. Resin dosages of 10, 50, 100, 250 and 500 g/L were tested. The bottles were left mixing at 1000 RPM and at 4^{o}C to prevent potential biological removal of COD. The removal of soluble COD from the liquid-phase was measured overtime in all conditions, and after 16 days of contact time the resulting equilibrium soluble COD concentrations were determined. The initial soluble COD concentration of the liquid-phase was of 3,882 mg/L. In addition to measuring removal of soluble COD, for the three candidate materials that had removed the most soluble COD, equilibrium concentrations of liquid-phase acetic, propionic, and butyric acid were also determined. The effect of these three materials on methane production was also evaluated by adding anaerobic sludge, collected from the continuous AnMBR system, to the serum bottles of each condition and measuring methane production over time under ambient temperature conditions. The anaerobic sludge was added at a dosage of 10% v/v so as not to contribute a significant amount of additional soluble COD. The bottles were sealed, and the head space was sparged with nitrogen gas to remove oxygen. The bottles were left mixing at 1000 RPM under

ambient temperature conditions (21°C). Batch test anaerobic digestion was carried out for 55 days with regular measurements of biogas production, using a water displacement column, and biogas quality to determine the benefit that each candidate material had on methane production.

Set-up and Operation of Continuous Two-phase AnMBR System

The same two-phase AnMBR system that was used in Chapter 1 was also used for this study, as shown in Figure 4.1. The acid-phase reactor consisted of a 2.5 L (working volume of 1.5 L) spinner flask (Bellco) with heating and mixing provided by a heated magnetic stir-plate combined with a magnetic stir-bar attached to an impeller inside the reactor. The methane-phase reactor consisted of a 14 L (working volume 12 L) New Brunswick BioFlo 115 bioreactor. The BioFlo control unit provided mixing and control of temperature, pH and liquid levels inside the methane-phase reactor. Default settings for mixing and pH were 120 RPM and 7.4, respectively. Automatic additions of 10M NaOH via the BioFlo control unit were made to maintain pH in the methane-phase reactor. The methane-phase was maintained at ambient temperature (21°C), while temperature in the acid-phase was maintained at mesophilic conditions (37°C). A wet tip gas meter (www.wettipgasmeter.com) was installed to measure methane production.

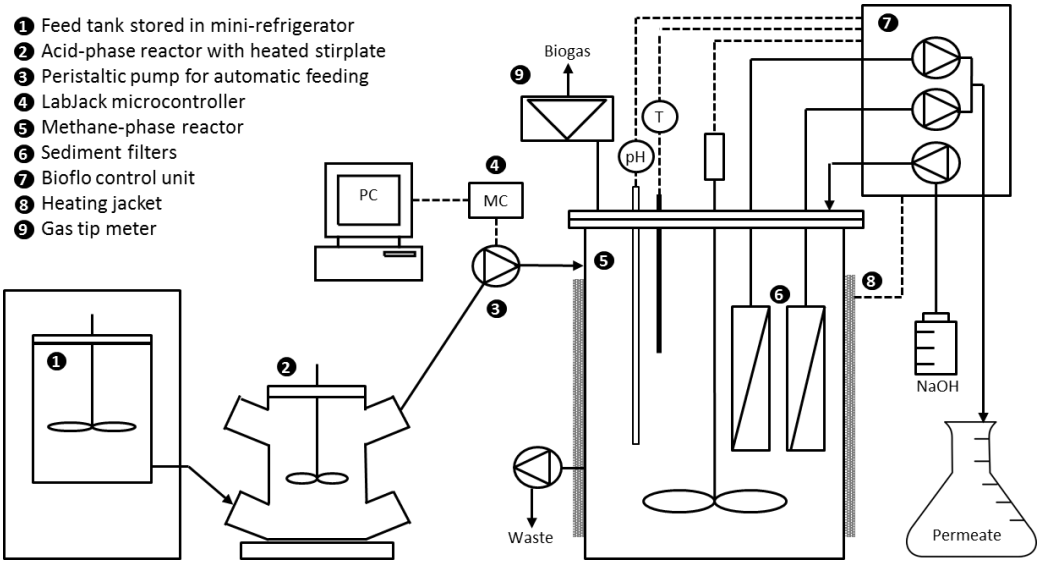


Figure 4.1. Schematic of two-phase continuous AnMBR system.

The methane-phase was initially seeded with mesophilic anaerobic sludge collected from the primary anaerobic digester of the UCSD Northeast Wastewater Treatment Plant. Automatic feeding in the system was carried out via a computer Python script used to command a Labjack

U3 DAC which controlled a Masterflex LS 07523-40 pump that intermittently pumped liquid from the acid-phase reactor to the methane-phase reactor to maintain the desired flow rate of 0.75 L/day. Upon pumping material between the acid- and methane-phase reactors, new influent was drawn into the acid-phase reactor via gravity from a stirred and refrigerated influent storage tank (4^{o}C). The Bioflo unit controlled the pumping of effluent out of the system through the microfiltration membranes inside the methane-phase reactor. HRT and SRT in the acid-phase were maintained at 2 days. HRT in the methane-phase was 14 days with an SRT of 30 days. The total organic loading rate (OLR) was maintained at 2.2 g COD/L d^{-1}. Table 4.3 summarizes operating conditions in the acid- and methane-phase of the AnMBR system.

Table 4.3. Operating conditions in the AnMBR system.

Parameter	Units	Acid-phase	Methane-phase
OLR	g COD/L d^{-1}	8.03 ±0.5	1.09 ±0.2
HRT	Days	2	16
SRT	Days	2	30
Temperature	°C	37 ±3	20±1
pH		5.3 ±0.5	7.6±0.1

As in Chapter 3, two 10 µm pore size, 0.11 m^2 cylindrical Omnifilter RS14-DS sediment filter cartridges served as the submerged membrane filtration component of the AnMBR system. To accommodate their placement in the methane-phase reactor, the filters were cut in half length wise and manifolded together. The filters had a final combined surface area of 0.258 m^2. To overcome fouling, sediment filters were replaced approximately every 50-100 days.

Addition of Ion-Exchange Resin to AnMBR and Organic Shock-load Testing

Upon achieving steady-state methane production in the continuous AnMBR system, the methane-phase was exposed to four organic shock-load events. The first shock-load to the system occurred on Day 127 and consisted of the addition of 16,000 mg COD/L of acetic acid to the methane phase of the AnMBR system. On Day 192, 1,200 g of TANEX resin (corresponding to a dosage of 100 g/L), distributed among three mesh bags (as shown in Figure 4.2), was added to the methane-phase reactor. Subsequently, a second shock loading event of 16,000 mg COD/L acetic acid was added to the system on Day 200. On Day 244 the TANEX resin was removed from the AnMBR reactor by removing the three mesh bags, and a third similar shock-load was

conducted on Day 256. The final shock-load was added on Day 321 after addition of a second, higher dosage of TANEX resin, 300 g/L, which this time was added freely to the reactor (i.e. no mesh bags). Soluble COD levels, methane production, and pH were all monitored during this time to determine the benefits of the addition of TANEX on system performance and recovery after shock-loading in terms of effluent quality and methane production.

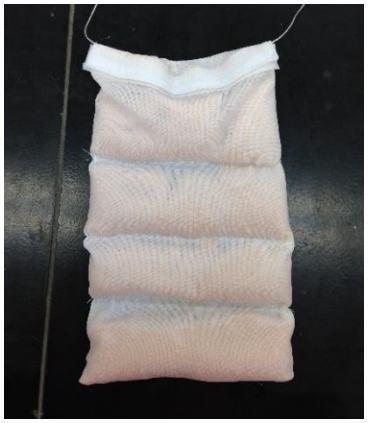


Figure 4.2. One of three mesh bags that were deployed into the continuous AnMBR system containing approximately 400 g of Purolite® TANEX™ each for a total dosage of 100 g/L.

Analytical Methods

All analytical methods were carried out according the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). Volatile fatty acid analysis was conducted by the Metabolmics Center at the University of Illinois at Urbana-Champaign using GCMS. Biogas samples were collected regularly from the AnMBR system and ambient batch test via syringe and 7 mL Vacutainer sample vials (BD Vacutainer, 8020128). Biogas quality was measured by gas chromatography (Varian, Model CP-3800), equipped with an Alltech Hayesep D 100/120 column and a thermal conductivity detector (TCD). The carrier gas was helium at a flow rate of 30 mL/min. Temperature of both the injector and detector was 120˚C.

Microbial Community Analysis

Samples from the methane-phase of the AnMBR system (25 ml) were collected before and after shock-loading events to investigate changes the microbial community. Specific sampling days were: 70, 88, 121, 179, 182, 194, 237, 240, 275, 297, 299, and 345. DNA

extraction from the samples was carried out using the FastDNA™ SPIN Kit for Soil (MP Biomedicals, LLC) using the manufacturer's instructions. The extracted DNA was stored at -20°C until submitted for sequencing. Sequencing of the extracted DNA was carried out by the University of Illinois Keck Center using Illumina Miseq sequencing combined with Fluidigm sample preparation. Primer pair V4-515F - V4-806R was used to amplify the V4 region of the 16S rRNA gene of bacteria, and primer pair ArchaeaF349 - ArchaeaR806 was used to amplify the 16S rRNA gene of archaea. Mothur version 1.35.0 (Schoss et al. 2009) was used to assemble the forward and reverse sequences using the standard operating procedure for Miseq data (Kozich et al. 2013 accessed January 2015). Sequences that appeared 3 times or less in the entire data set were removed. Alignment and taxonomic classification was done using the Silva Bacterial and Archaeal reference databases, release 102, as provided by Mothur (Schloss et al. 2009). Nonmetric multidimensional scaling (nMDS) plots, using Bray-Curtis dissimilarity index were generated using the software Primer-E Version 7 (Quest Research Limited, Auckland, New Zealand) to visualize changes among the microbial communities.

Results and Discussion

AnMBR performance under ambient temperature

Prior to this study, both phases of the AnMBR system had been operated at mesophilic temperature (37°C). Figure 4.3 shows the profiles of methane production and effluent COD in the AnMBR system during the initial transition from mesophilic to ambient temperature operation and start-up of this study on Day 0. The initial change to ambient temperature (21°C) in the methane-phase resulted in a significant decrease in methane production accompanied by an accumulation of undigested material, eventually leading to reactor failure with zero biogas production. It was at that point that the reactor was reseeded with fresh anaerobic sludge, and start-up for the current study began. After re-seeding the reactor on Day 0, average methane production under ambient temperature conditions stabilized around an average of 254 mL/g VS by Day 75. This was 39% less than average methane production under mesophilic conditions, which was 414 mg/L. The decreased methane production under ambient temperature can be explained by both a decrease in microbial activity at lower temperature, as well as an increase in dissolved methane. It is well known that the solubility of methane increases with decreasing temperature (Stuckey, 2012). An excepted value of dissolved methane can be estimated using

Henry's law. However, recent studies have found that this value is significantly lower than actual dissolved methane concentrations, and that supersaturation of dissolve methane can occur under ambient temperature operation. Hartley and Lant (2006) found that for a pilot-scale anaerobic migrating bed reactor treating sewage at ambient temperature, the ratio of actual dissolved methane due to supersaturation (C) relative to expected dissolved concentration (Ce) was between 0.8-2.2 C/Ce, with an average of 1.6. Applying these values to the current study, between 8-21% of measured gas-phase methane production could have been dissolved methane during ambient temperature operation. With that in mind, it will be important to consider methods for recovering dissolved methane under ambient temperature operation, so that the potent greenhouse gas is not released into the atmosphere, and to achieve greater energy recovery.

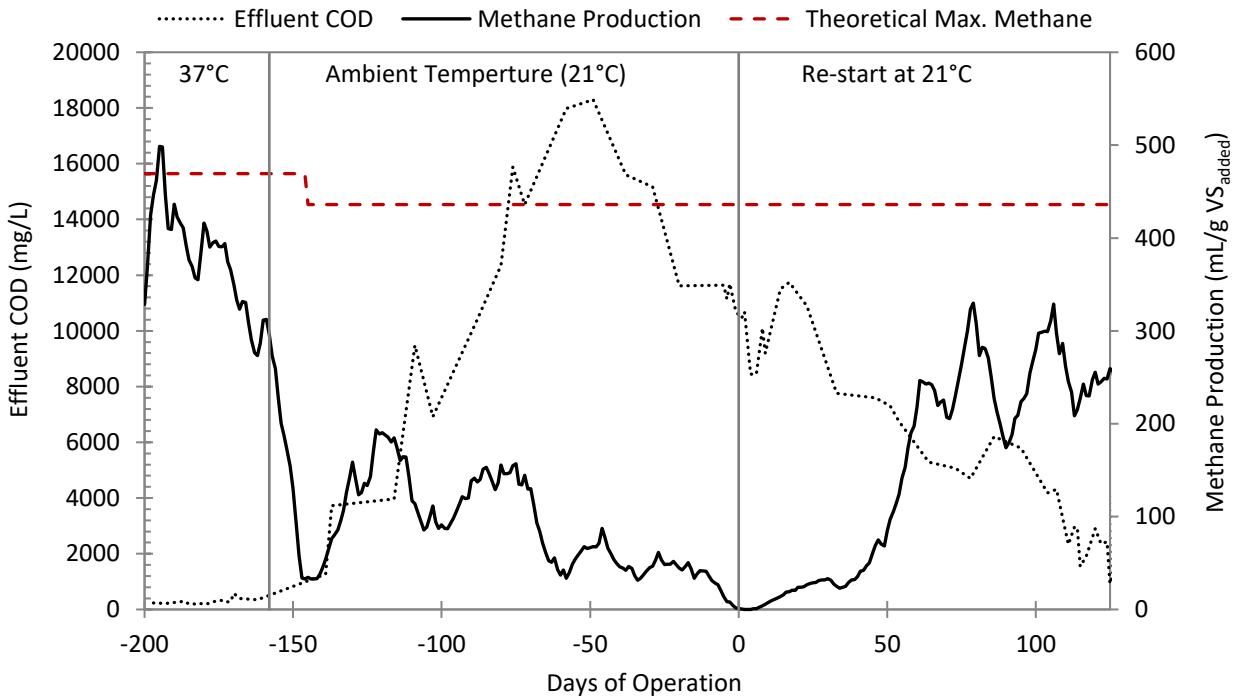


Figure 4.3. Methane production and COD concentrations in the methane-phase of the AnMBR system during the transition from mesophilic to ambient temperature operating conditions, and after re-seeding with fresh anaerobic sludge (Day 0).

The upset to reactor performance caused by the initial temperature drop led to an accumulation of undigested organics in the AnMBR reducing effluent quality, as indicated by elevated effluent COD concentrations as shown in Figure 4.3. However, as reactor performance under ambient temperature stabilized and conversion of organic material to methane improved, effluent quality similarly improved and effluent COD stabilized between 1000-2000 mg/L. This was approximately an order of magnitude higher than average effluent COD concentrations under mesophilic conditions (247 mg/L). Overall, decreasing the AnMBR operating temperature from mesophilic to ambient conditions negatively impacted reactor performance leading to accumulation of undigested organic material, reduced methane production, and loss of valuable soluble COD in the effluent. Thus, the addition of an ion-exchange resin as a means for minimizing such a reactor upset as well as improving reactor recovery time and effluent quality in the event of organic shock-loading was investigated.

Batch-test screening of candidate ion-exchange resins for COD and VFA removal

Initial batch test screening of four different candidate ion-exchange materials, as well as GAC, was conducted to identify the best material for deployment in the continuous AnMBR system. Candidate materials were first evaluated for their ability to remove soluble organics, particularly VFAs, from AnMBR effluent. The five materials were added to AnMBR effluent in dosages ranging from 10 g/L to 500 g/L. After 16 days of contact time, the resulting equilibrium liquid-phase sCOD concentrations were compared to determine the ability of each adsorbent material to remove excess soluble organics from the liquid-phase. The resulting equilibrium soluble COD concentrations for the different materials and dosages are shown in Figure 4.4. These results indicated that GAC provided the best removal of soluble COD followed by the two strong-base anion exchange resins: Purolite TANEX and Purolite A510, while the two weak-base anion exchange resins: Purolite A845 and Purolite A830, provided the least removal.

The top three COD removing materials, GAC, Purolite TANEX, and Purolite A510, were then evaluated for their ability to remove VFAs. Figure 4.5 shows the concentration of acetic, propionic, and butyric acid remaining in the liquid-phase for dosages of 10, 100, and 250 g/L of each candidate material. Purolite TANEX was found to provide the greatest removal of all three VFAs, followed by the other strong-base anion-exchange resin, Purolite A510. GAC resulted in the lowest removal of VFAs.

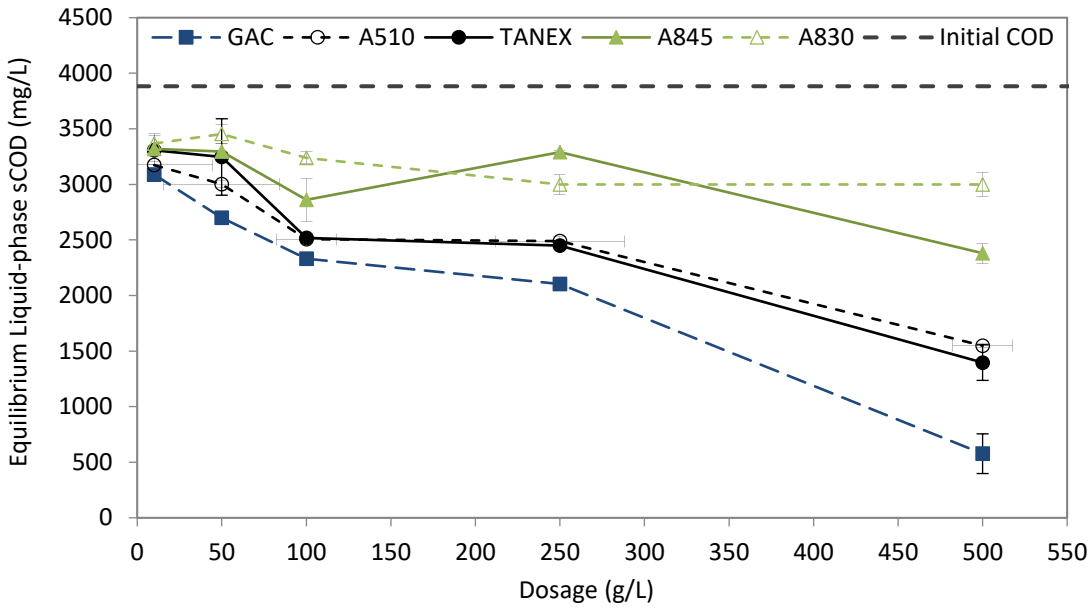


Figure 4.4. Equilibrium liquid phase COD concentrations in batch adsorption testing of for various dosages of GAC and candidate ion-exchange resins (10, 50, 100, 250 and 500 g/L) after 16 days of contact time in filtered AnMBR effluent (initial liquid phase COD = 3882 mg/L).

In addition to soluble organics removal, the effect of the various candidate materials on methane production was also investigated by combining the loaded materials (GAC, TANEX, and A510) with anaerobic sludge and monitoring methane production over time. Figure 4.6 shows methane production for each condition after 40 days of batch digestion under ambient temperature conditions. It can be seen that the strong-base anion-exchange resins at dosages of 10 and 100 g/L, resulted in the greatest methane production. The 250 g/L resin conditions produced significantly less methane, possibly related to the higher pH levels that were observed in these conditions. The legend in Figure 4.6 includes the measured pH value for each condition on the last day of digestion. GAC conditions resulted in the lowest methane production. Since all conditions received the same amount of organic feed material, this could suggest that in the case of GAC, the adsorbed organics were less accessible for microbial degradation. Access to the soluble substrates could also have been slowed by the highest doses of resin, which may have contributed to reduced methane production during the experimental time frame. Based on improved batch test removal of COD and VFA, as well as higher methane production, Purolite TANEX was determined to be the best material for further testing in the continuous AnMBR system. An initial dosage of 100 g/L of reactor volume was chosen to be applied in the AnMBR system.

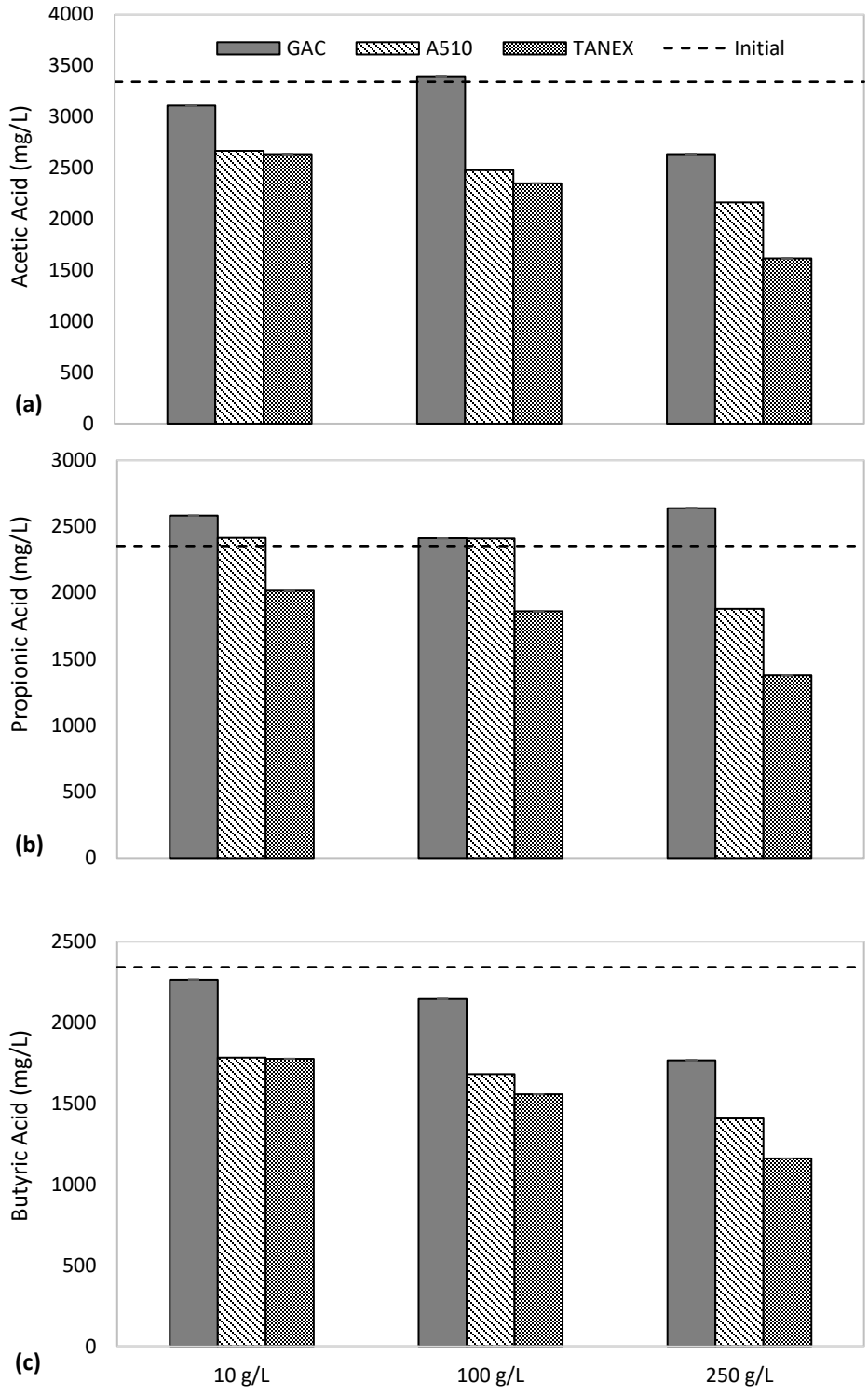


Figure 4.5. Equilibrium liquid-phase concentrations of volatile fatty acids (a) acetic acid, (b) propionic acid, and (c) butyric acid, in batch adsorption testing of various dosages (10, 100 and 250 g/L) of GAC, Purolite® A510 and Purolite® TANEX™ resin after 16 days of contact time in filtered AnMBR effluent.

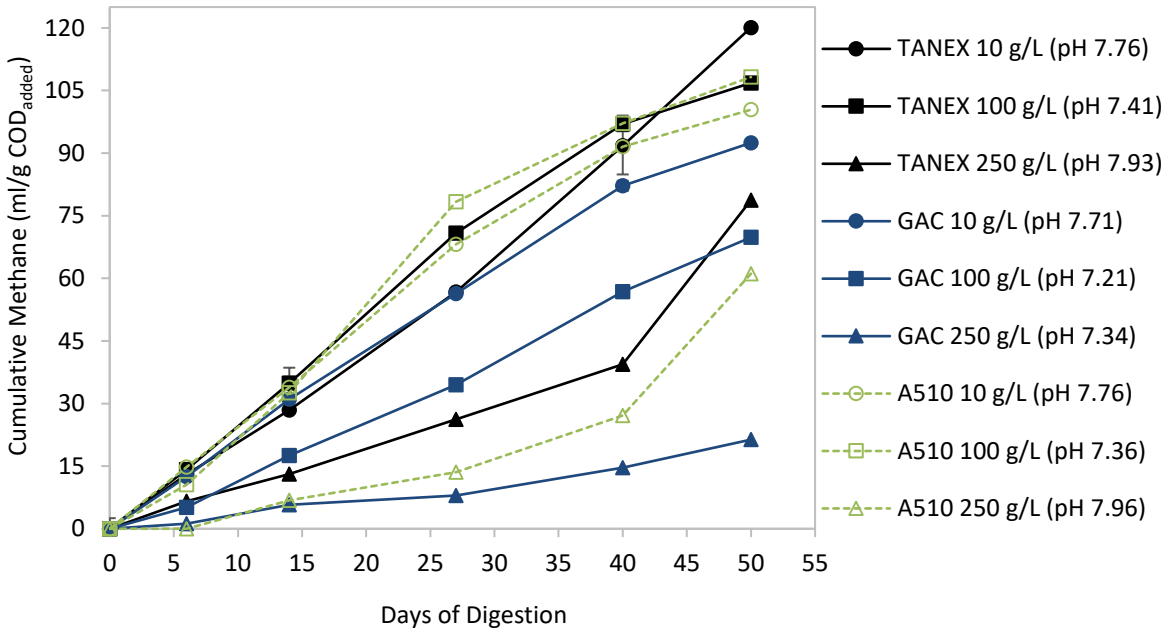


Figure 4.6. Methane production per gram of COD_{added} from batch test anaerobic digestion of used adsorbent materials (GAC, TANEX, and A510) dosages (10, 100 and 250 g/L).

AnMBR performance after shock-loading with and without TANEX resin

After identifying Purolite TANEX to be the best candidate material, reactor performance under organic shock-loading conditions was investigated with and without the addition of the TANEX resin. Figure 4.7 provides an overview of the sequence of shock-load events that occurred in the AnMBR system over the course of the study, showing methane-phase soluble COD and effluent COD concentrations over time in Figure 4.7a, and methane production over time in Figure 4.7b. The first shock-load to the system occurred on Day 122. After recovery from that shock-load, 100 g/L of TANEX resin was added to the methane-phase reactor on Day 188, and a second shock-load was applied on Day 196. On Day 240, the resin was removed and a third shock-load was applied on Day 251. Finally, on Day 314, 300 g/L of TANEX resin was added to the reactor and a final shock-load was applied on Day 316. Addition of 100 g/L TANEX on Day 188 provided an immediate benefit, reducing soluble COD levels in the methane-phase reactor by 60%, from 4246 mg/L to 1687 mg/L, and reducing effluent COD levels by 48% from 3218 to 1674 over the first 3 days of deployment. Effluent COD remained below 2105 mg/L until the second shock-load event. Similarly, the second addition of TANEX resin, this time at a dosage of 300 g/L on Day 314, decreased methane-phase soluble COD by

48%, from 1554 to 812, and effluent COD concentrations decreased by 75%, from 1542 to 382 mg/L. Thus, the addition of TANEX resin in the AnMBR system had a beneficial effect on effluent quality via sorption of excess soluble COD.

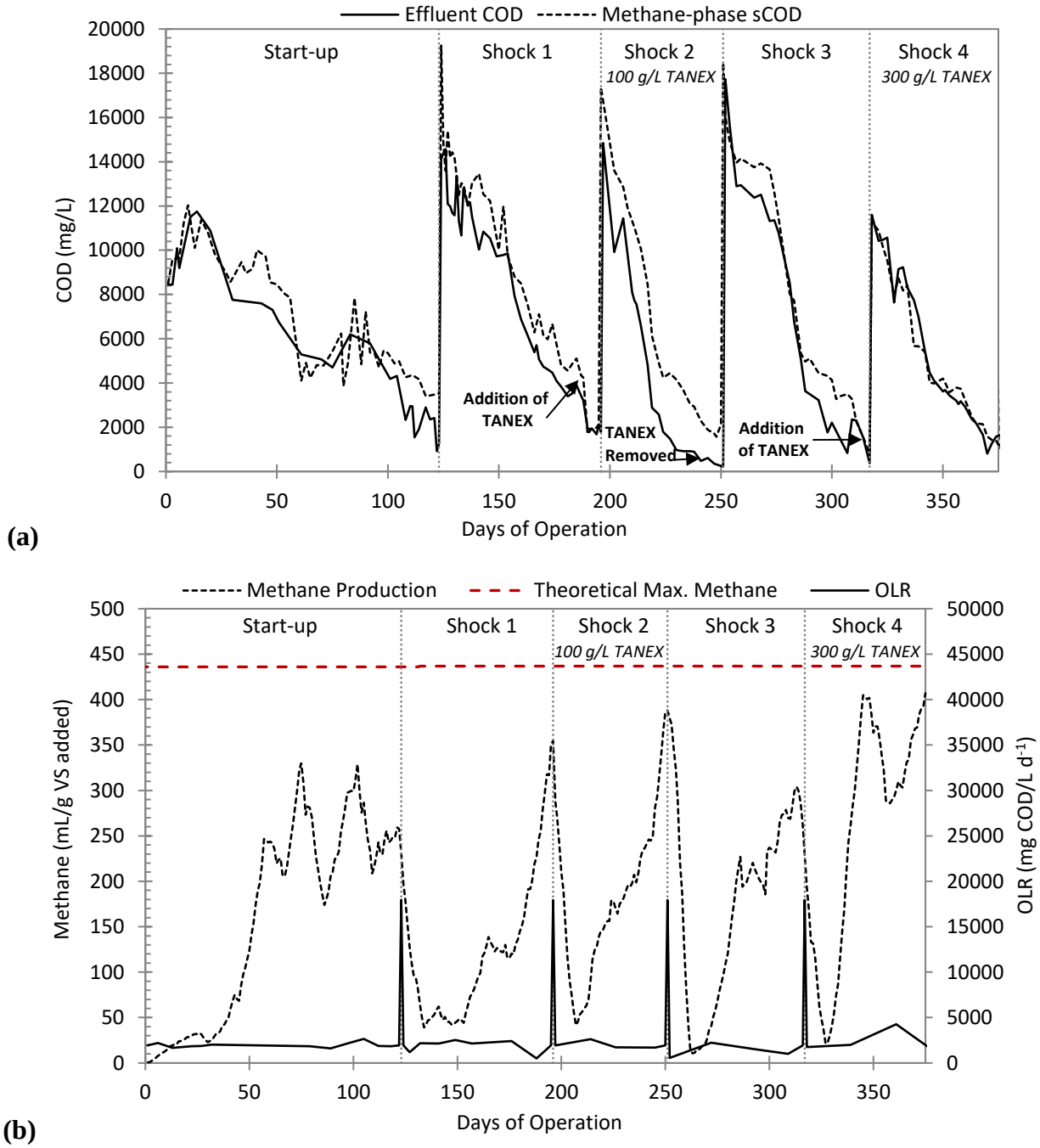


Figure 4.7. (a) Effluent COD and (b) methane-production in AnMBR system during all periods of operation with and without the addition of Purolite® TANEX™ under ambient temperature operation.

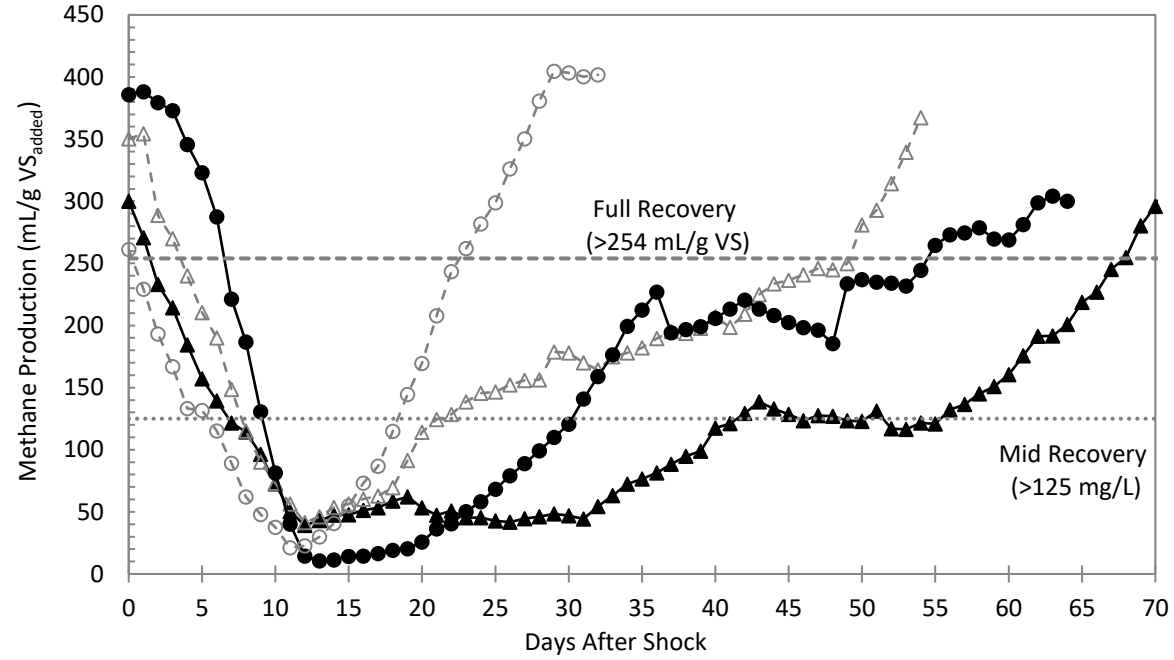
Looking at methane-production, all shock-load events resulted in a severe initial drop in methane production, likely due to the decrease in pH that was associated with the addition of 16,000 mg COD/L of acetic acid. During all shock-load events pH control was turned off for the first 5 days to investigate the possible benefit of the TANEX resin on mitigating pH fluctuations as a result of organic shock-loading. However, in all cases, with and without the TANEX resin, pH in the AnMBR dropped sharply upon shock-loading, from 7.4 to 4.7-5.3, with little increase during following 5 days without pH control. This was somewhat surprising in the case of shock-loading with the addition of the TANEX resin, since it was expected that the exchange of hydroxide ion for acetate would help to stabilize pH. Evidently, many of the hydroxide ions had already exchanged for other ions prior to the shock-loading event. This finding motivated the application of a higher dosage of resin, 300 g/L of reactor volume in the continuous system. However, pH after the fourth shock-load with the addition of 300 g/L TANEX still fell to 4.7 and only increased to 5.0 within the 5 days after shock-loading. Thus, it is possible that for the given organic shock-load, a much higher dosage of TANEX resin is needed in order to buffer the pH drop associated with the addition of such a high concentration of acetic acid. In addition, it is possible that some HCO_3^- was sorbed onto the resin thus reducing the alkalinity in the reactor.

Nevertheless, although addition of TANEX resin did not mitigate the pH change associated with the organic shock-load, after re-establishing automatic pH control, reactor recovery in terms of both methane production and effluent quality was faster during the periods of operation with the addition of TANEX resin than without it. Figure 4.8 shows methane production and effluent COD in the AnMBR system after each shock-load event. Methane production is shown in Figure 4.8a. Full recovery of methane production was defined as production greater than >254 mL/g of VS_{added} which was the average methane production achieved after start-up, before shock-loading. Mid-recovery was defined as methane production >125 mL/g VS_{added}. From Figure 4.8a, it can be seen that the first shock-load without TANEX had the longest full recovery time of 68 days, with mid-recovery after 42 days. Adding 100 g/L of TANEX to the system improved recovery time by 26%, achieving mid-recovery in 21 days and full recovery in 50 days. An interesting thing happened during recovery from the third shock-load (second shock without resin). In this case, initial methane recovery was slower than the recovery with TANEX resin present, achieving mid recovery after 30 days compared to 21. However, full recovery was achieved within 55 days after Shock 3, which was 19% faster

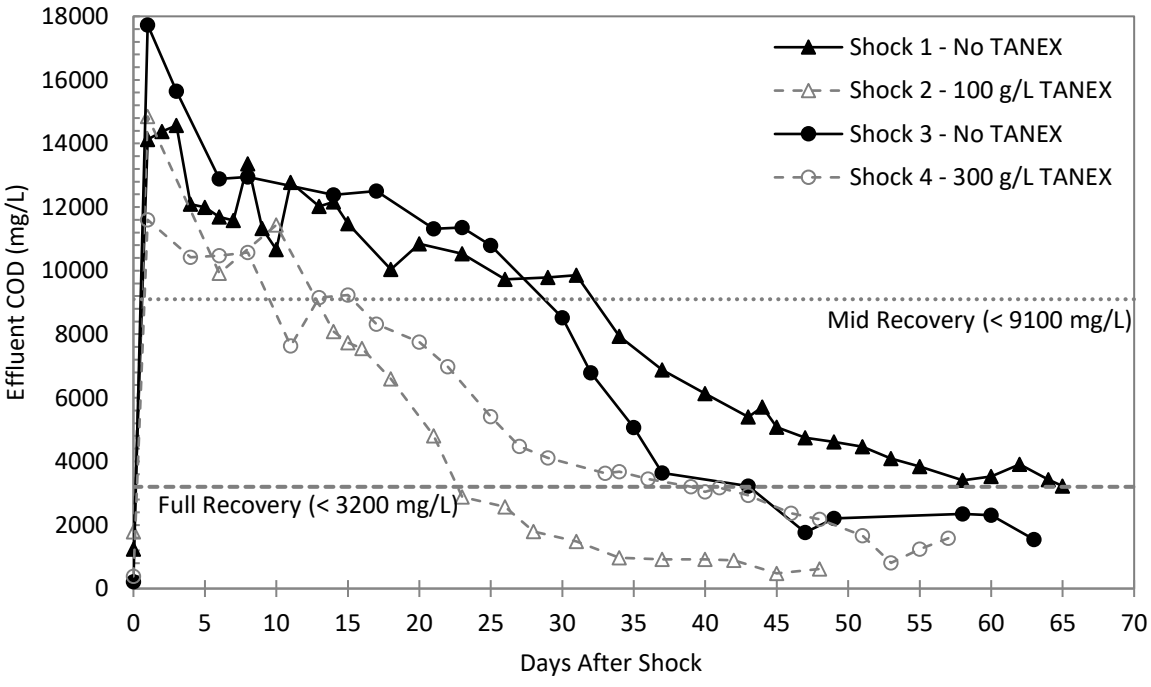
recovery after the first shock without the addition of TANEX and only 9% slower than recovery with the addition of 100 g/L TANEX. Thus, there was an improvement in reactor recovery over time when comparing the two shock loading events without the addition of TANEX resin. The improvement suggests that some acclimation of the microbial community occurred, making it better able cope with the increased acetic acid levels. Such acclimation has been seen in other studies, where the population of *Methanosarcina* was found to increase with higher concentrations of acetic acid present (Mosbaek et al, 2016; Pamasiri et al, 2007; Venkiteshwaran et al, 2016). Although reactor recovery after shock-loading improved without addition of TANEX resin, recovery times with the addition of TANEX resin were still faster overall. Thus, the benefits of adding resin were greater than the benefits of acclimation when comparing the Shock 2 and Shock 3 events. Full recovery with the addition of 100 and 300 g/L of TANEX resin was 9 and 58% faster, respectively, than recovery after Shock 3 without addition of the resin. Thus, the higher dosage of TANEX resin, applied at the start of Shock 4, resulted in the fastest recovery of methane production achieving full recovery after just 23 days.

Recovery of effluent quality was also faster with the addition of the TANEX resin than without. The profile of effluent COD concentrations after all four shock-loading events is shown in Figure 4.8b. Full recovery was defined as achieving effluent COD levels less than 3200 mg/L, which was the lowest effluent soluble COD concentration achieved after the first shock-load event without addition of TANEX resin. Mid recovery was defined as less than 9100 mg/L. As can be seen in Figure 8b, full recovery of effluent COD levels after shock-loading was up to 65% faster with the addition of TANEX resin compared to without. Without TANEX, full recovery took between 43 and 65 days, with mid-point recovery around 29-33 days. Addition of TANEX reduced mid-point recovery time by 55-61%, achieving mid-point recovery around 13 days, and full recovery within 23 and 40 days with the addition of 100 and 300 g/L TANEX, respectively. Again, an improvement between the two shock-loads without the addition of TANEX resin (Shock 1 and 3) was observed reflecting acclimation of the microbial community. Full recovery of effluent COD quality was 34% faster after Shock 3 than Shock 1, this was similar to the recovery with 300 g/L of TANEX resin during Shock 2. Effluent COD directly after the fourth shock-load, with the addition of 300 g/L TANEX, was much lower than that after the previous three shock-loads, likely owing to the higher dosage of TANEX resin. To completely mitigate increased effluent COD concentrations due to such a large organic shock-load, a higher dosage

of TANEX resin would be required. Alternatively, it may be worth evaluating in future work if adding some cationic resin could be used to help buffer against sudden deceases in pH caused by hydrogen ions dissociating from VFAs.



(a)



(b)

Figure 4.8. An overlay of recovery of (a) methane production and (b) effluent COD, to pre-shock load levels in the continuous AnMBR after four organics shock-loading events (addition of 16,000 mg/L acetic acid) with and without the addition of Purolite® TANEX™ resin.

Microbial Community Analysis

Microbial community analysis via Illumina MiSeq 16S rRNA gene sequence analysis was carried out to investigate changes in the methane-phase microbial community over the course of the study. A total of 1988 distinct bacterial OTUs and 383 distinct archaeal OTUs were identified in samples collected from the methane-phase near the end each period of operation. Using the relative abundances of these OTUs, non-metric multidimensional scaling plots were generated based on the Bray-Curtis Similarity Index to visualize the similarity/dissimilarity of the microbial communities that had evolved toward the end of each operating period. Figure 4.9a is an nMDS plot of bacterial communities. Samples collected from the start-up period of the reactor, before shock-loading, grouped most similarly with 55.8% similarity. Grouping among the samples collected after the four different shock-load events was weaker. The majority of samples collected from shock-loaded conditions grouped within 35% similarity. Thus, it was apparent that shock-loading did have an effect on the bacterial community since all samples prior to shock-loading grouped similarity to each other and separate from samples collected after shock-loading. However, there was not a clear shift within the shock-loaded samples toward a similar bacterial community. In addition, the presence of TANEX resin did not have a controlling effect on the microbial community because the samples taken with and without resin present did not separate into distinct regions in the Bray-Curtis analysis.

Looking at the archaeal community in Figure 4.9b, a clearer shift was observed between start-up and shock-load microbial communities. All start-up samples grouped within 35.8% similarity, and the majority of shock-load samples grouped separate from start-up samples within 34.8% similarity. The exception was the last sample collected after Shock 1, which fell outside of both the start-up and shock-load groups. Thus, the archaeal community that developed after Shock 1 was the least similar to both start-up and later shock-load archaeal communities.

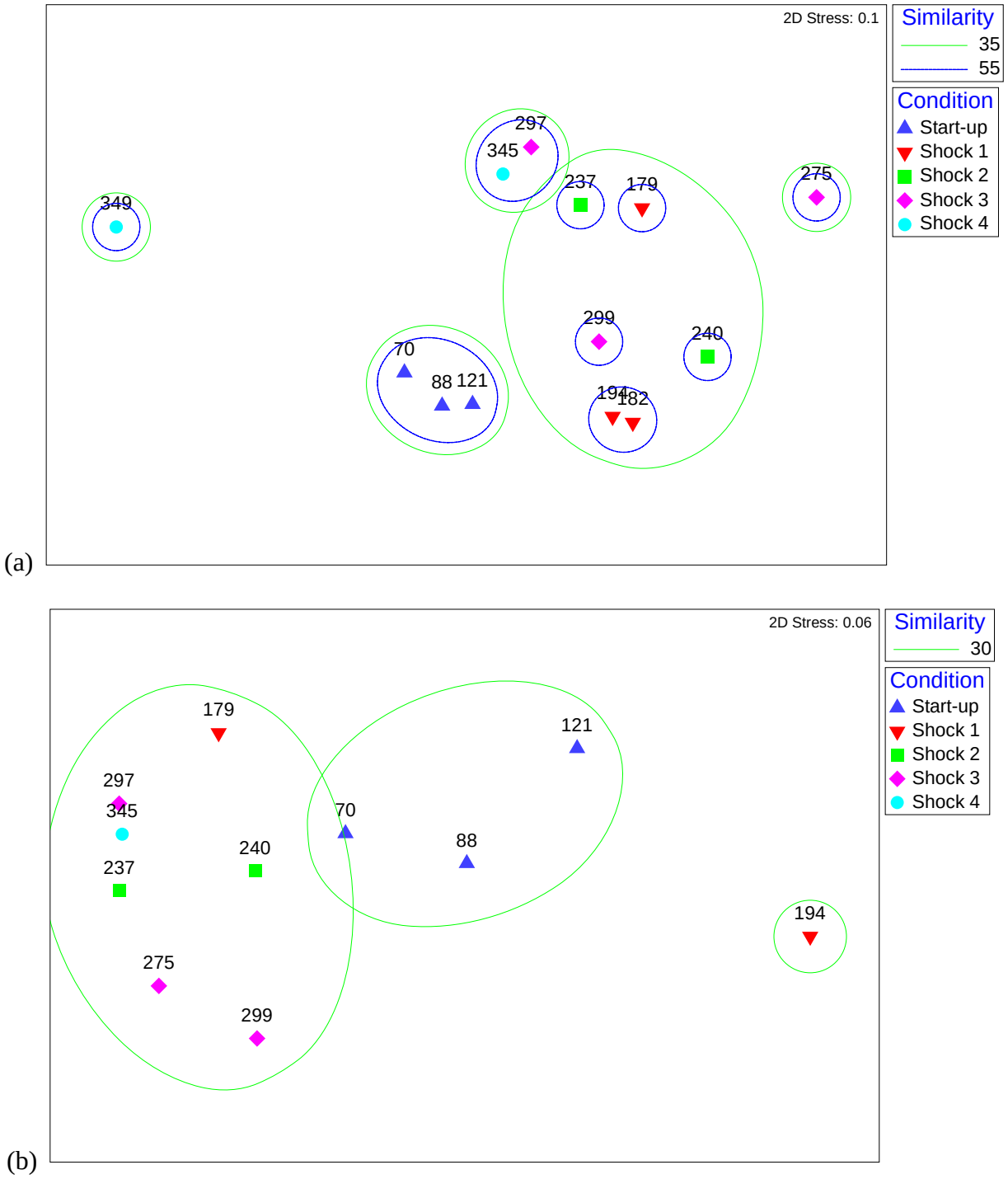


Figure 4.9. Bray-Curtis Similarity Index nMDS plots of (a) bacterial and (b) archaeal communities sampled during each period of operation including, start-up, and after each shock-load event (Shock 1-4) labeled by sample day.

Looking at specific archaeal genera identified within the different sampled microbial communities, the most pronounced difference between start-up and Shock 1 communities was a decrease in the relative abundance of *Methanosarcina* related OTUs. Figure 4.10a shows the relative abundance of the different archaeal genera identified within the samples, with average values summarized in Table 4.4. *Methanosarcina* was the most abundant archaeal genus during start-up making up 27.6% of relative abundance. After Shock 1 the average relative abundance of *Methanosarcina* decreased to 18.7%. In the following periods of operation, the relative abundance of *Methanosarcina* increased 1.5-2 times what is was during start-up, with a relative abundance of 58.1, 41.2, and 55.3% during Shock 2, 3 and 4, respectively. *Methanosarcina* has been identified as important group of methanogens in anaerobic digestion due to their relatively low doubling times and robustness towards different process impairments including high VFA concentrations (Ali Shah et al 2014; Ketheesan and Stuckey, 2015; Vrieze et al 2012). They are also one of the only two methanogenic genera known to consume acetate, the other being *Methanosaeta* (Liu and Whitman, 2008). Thus, it makes sense that the relative abundance of this group of archaeal organisms would increase in response to increased acetic acid levels. Previous studies have also reported increased relative abundance of *Methanosarcina* due to acetate accumulation. Venkiteshwaran et al (2016) investigated the performance and microbial community structure of batch digesters seeded with different inoculum and found that high relative abundance of *Methanosarcina* correlated to higher methane production and lower effluent acetate concentrations. Similarly, Padmasiri et al (2007) found that high and low levels of acetate in an AnMBR treating swine manure corresponded to increased relative abundance of *Methanosarcinaceae* and *Methanosaetacea* respectively. Finally, Mosbaek et al (2016) investigated microbial community changes in batch test digesters after recovery from volatile fatty accumulation concluding that *Methanosarcina*, as well as *Methaneoculleus*, and five different acetate-consuming subspecies of Clostridia were involved in the turnover of accumulated acetate. Figure 4.10b shows the relative abundance of the different bacterial phyla identified in the samples during each operating period, with average values summarized in Table 4.5. The most notable difference among shock-loaded samples compared to the start-up samples was a decrease in the relative abundance of Bacteroidetes and an increase in the relative abundance Firmicutes. Relative abundance of Firmicutes were highest (19.1 and 22.7%) during

the two shock-loads with TANEX (Shock 2 and 4). This may reflect an increase in Clostridia or other acetate-consuming organisms as was observed in the study by Mosbaek et al (2006).

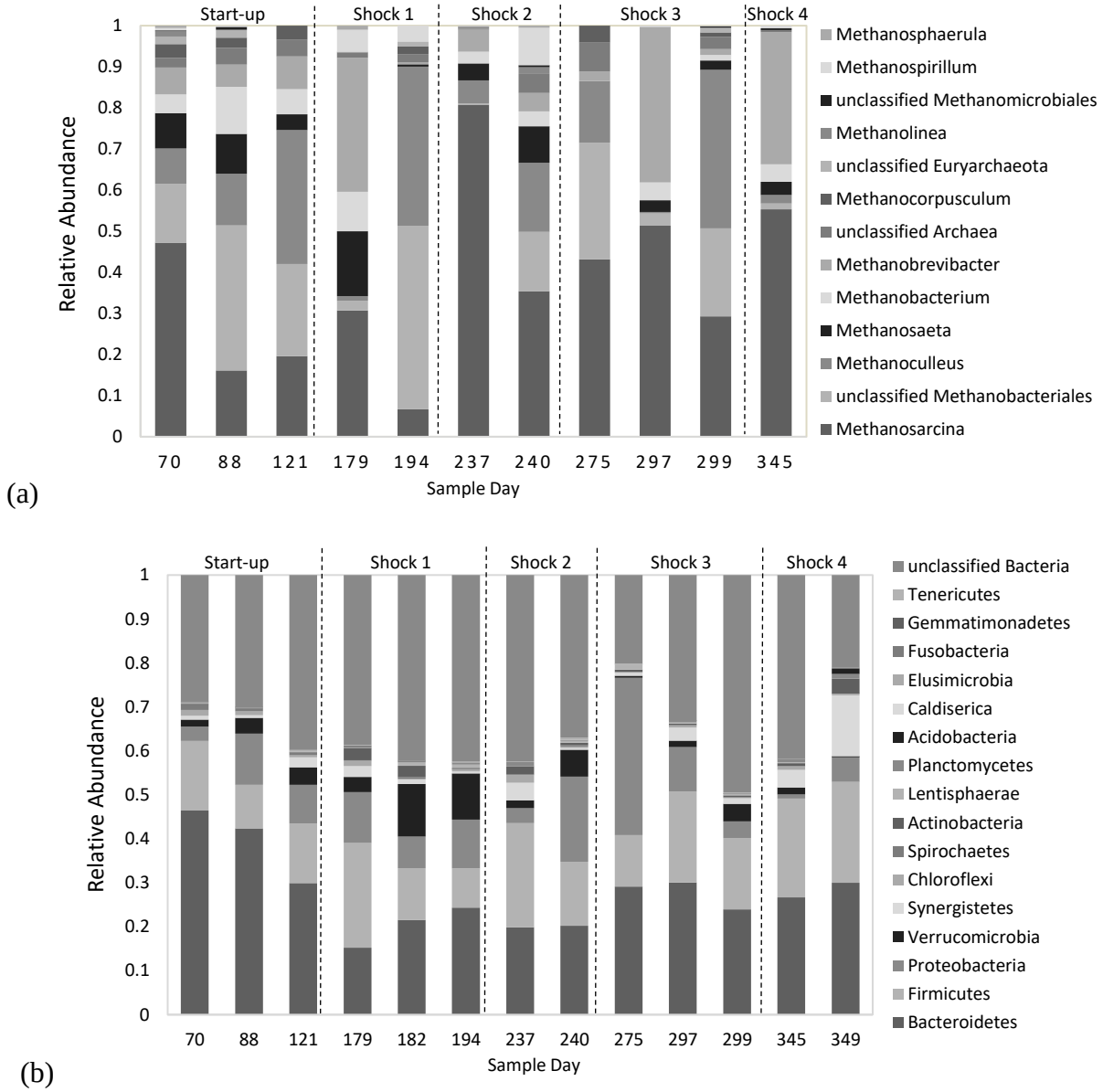


Figure 4.10. Relative abundance of (a) archaeal and (b) bacterial OTUs, identified by genus and phylum level respectively, in the methane-phase reactor during each period of operation.

Table 4.4. Average relative abundance of archaeal genera identified in the methane-phase during each period of operation.

Genus	Average Relative Abundance (%)				
	Start-up	Shock 1	Shock 2	Shock 3	Shock 4
Methanosarcina	27.6	18.7	58.1	41.2	55.3
unclassified Methanobacteriales	24.0	23.5	7.4	17.6	1.4
Methanoculleus	17.9	19.9	11.2	18.0	2.0
Methanosaeta	7.4	8.2	6.5	1.7	3.2
Methanobacterium	7.4	4.8	3.2	1.9	4.3
Methanobrevibacter	6.6	16.5	4.9	13.8	32.2
unclassified Archaea	3.1	1.7	2.1	2.7	0.4
Methanocorpusculum	3.0	1.0	0.1	1.8	0.0
unclassified Euryarchaeota	1.3	0.6	0.1	0.4	0.0
Methanolinea	0.4	0.1	0.8	0.0	0.0
unclassified Methanomicrobiales	0.3	0.0	0.3	0.1	0.4
Methanospirillum	0.3	4.6	4.7	0.1	0.7
Methanosphaerula	0.2	0.5	0.3	0.0	0.0

Table 4.5. Average relative abundance of bacterial phyla identified in the methane-phase during each period of operation.

Phylum	Average Relative Abundance (%)				
	Start-up	Shock 1	Shock 2	Shock 3	Shock 4
Bacteroidetes	39.6	20.4	20.1	27.7	28.4
Firmicutes	13.1	14.8	19.1	16.2	22.7
Proteobacteria	7.9	10.0	11.4	16.6	3.3
Verrucomicrobia	3.1	8.6	4.0	2.0	1.0
Synergistetes	1.2	1.3	2.2	1.7	8.9
Chloroflexi	0.9	0.6	1.0	0.2	0.6
Spirochaetes	0.8	0.2	0.2	0.1	0.0
Actinobacteria	0.2	1.9	1.2	0.4	2.0
Lentisphaerae	0.1	0.5	0.3	0.5	0.1
Planctomycetes	0.1	0.3	0.5	0.2	0.9
Acidobacteria	0.0	0.1	0.0	0.0	0.6
Caldiserica	0.0	0.0	0.0	0.0	0.0
Elusimicrobia	0.0	0.1	0.1	0.0	0.0
Fusobacteria	0.0	0.0	0.0	0.0	0.0
Gemmatimonadetes	0.0	0.0	0.0	0.0	0.1
Tenericutes	0.0	0.1	0.1	0.1	0.0
unclassified Bacteria	32.9	41.1	39.7	34.2	31.5

In this study, the observation that Shock 1 archaeal communities were different than the start-up community but not as similar to later shock-load communities, and the fact that this condition experienced the slowest recovery, suggests that the microbial community had not acclimated to increased VFA loads at this point. Acclimation of the microbial community seemed to have occurred by the end of Shock 2, with the increase in *Methanosarcina* and Firmicutes relative OTUs. In addition, the relative abundance of *Methanosarcina* was highest during the two periods of operation with the TANEX resin. Thus, it is believed that addition of TANEX during Shock 2 benefited acclimation of the microbial community to better tolerate high acetic acid conditions. The lower initial effluent COD concentrations seen after both shock-loading events with the addition of TANEX compared to without suggests that the TANEX resin provided a reservoir for the excess soluble COD that was added to the system. By this means, the TANEX would have provided relief to the microbial community, allowing it time to adjust and become better able to process the higher levels of acetic acid. Comparing effluent soluble COD concentrations between Shock 2 and 3, it can be seen that without the sorption of soluble COD provided by the TANEX resin during Shock 2, higher levels of soluble COD escaped the system in the effluent after Shock 3, despite improved recovery compared to Shock 1. Thus, although a more efficient microbial community seemed to have developed between the time of Shock 1 and 3, effluent COD levels were still not as low as that during Shock 2 and 4 when the TANEX resin was present. Therefore, the addition of TANEX resin provided a clear benefit to effluent quality and process recovery after organic shock-loading.

Conclusions

Application of a strong-base anion-exchange resin, Purolite TANEX, in a two-phase continuous AnMBR process was investigated as a means for improving process performance under ambient temperature operation and reactor recovery after organic shock-loading. Initially, four candidate anion-exchange resins were evaluated based on their ability to adsorb soluble COD and VFAs, as well as their benefit on methane production during batch testing. Purolite TANEX proved to be the best candidate resin for sorption of VFAs and improving methane production. Based on these results, TANEX was deployed in the continuous AnMBR system. Subsequently, reactor stability and recovery after shock-loading with and without the addition of TANEX was investigated. Results indicated that addition of 100 and 300 g/L of the resin to the AnMBR system provided an initial benefit of reduced effluent COD concentrations from 3218 to

1674 mg/L and from 1542 to 382 mg/L, respectively. Upon shock-loading, the presence of the resin also reduced recovery times, under controlled pH conditions. Specifically, the rate of recovery to pre-shock effluent COD levels was up to 65% faster and recovery of methane production was up to 58% faster. For the large organic shock load used in this study, 16,000 mg COD/L of acetic acid, the addition 100 and 300 g/L of TANEX resin was not able to fully mitigate the associated drop in pH. Thus, the benefits of TANEX as a buffer against pH changes needs further development. It is suspected that for lower concentration shock-loads, addition of the TANEX resin could potentially mitigate pH changes. Finally, microbial community analysis via 16S Illumina MiSeq sequencing revealed an increase in the relative abundance of *Methanoscarcina* and Firmicutes in the majority of post-shock-load microbial communities. The highest relative abundance of both *Methanoscarcina* (51-58%) and Firmicutes (19-23%) was seen during operating periods with the addition of TANEX resin, leading to the conclusion that addition of the TANEX resin provided relief to the microbial community via sorption of excess acetic acid thereby allowing time for acclimation and better ability to process the higher levels of acetic acid associated with the organic shock. Overall, addition of the anion-exchange resin TANEX provided a benefit to reactor performance and recovery after organic shock-loading.

References

1. Akram, A. (2007). *Stability and performance improvement of a submerged anaerobic membrane bioreactor (SAMBR) for wastewater treatment*. PhD Dissertation. Imperial College London.
2. Akram, A., & Stuckey, D. C. (2008). Flux and performance improvement in a submerged anaerobic membrane bioreactor (SAMBR) using powdered activated carbon (PAC). *Process Biochemistry*, *43*(1), 93–102.
3. Ali Shah, F., Mahmood, Q., Maroof Shah, M., Pervez, A., & Ahmad Asad, S. (2014). Microbial ecology of anaerobic digesters: The key players of anaerobiosis. *The Scientific World Journal*, *2014*.
4. Baloch, M. I., & Akunna, J. C. (2003). Effect of rapid hydraulic shock loads on the performance of granular bed baffled reactor. *Environmental Technology*, *24*(3), 361–368.
5. Chang, S. (2014). Anaerobic Membrane Bioreactors (AnMBR) for Wastewater Treatment. *Advances in Chemical Engineering and Science*, *4*(1), 56–61.
6. Da Silva, A. H., & Miranda, E. A. (2013). Adsorption/desorption of organic acids onto different adsorbents for their recovery from fermentation broths. *Journal of Chemical and Engineering Data*, *58*(6), 1454–1463.
7. De Vrieze, J., Hennebel, T., Boon, N., & Verstraete, W. (2012). Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. *Bioresource Technology*, *112*, 1–9.
8. Gujer, W., & Zehnder, A. J. B. (1983). Conversion processes in anaerobic digestion. *Water Science and Technolgy*, *15*(8–9), 127–167.
9. Hu, A. Y., & Stuckey, D. C. (2007). Activated Carbon Addition to a Submerged Anaerobic Membrane Bioreactor: Effect on Performance, Transmembrane Pressure, and Flux. *Journal of Environmental Engineering*, *133*(1), 73–80.
10. Hartley, K., Lant, P. (2006). Eliminating non-renewable CO2 emissions from sewage treatment: an anaerobic migrating bed reactor pilot plant study, *Biotechnol. Bioeng*. 95:384–398.
11. Ketheesan, B., & Stuckey, D. C. (2015). Effects of Hydraulic/Organic Shock/Transient Loads in Anaerobic Wastewater Treatment: A Review. *Critical Reviews in Environmental Science and Technology*, *45*(24), 2693–2727.
12. Ketheesan, B., & Stuckey, D. C. (2015). Effects of Hydraulic/Organic Shock/Transient Loads in Anaerobic Wastewater Treatment: A Review. *Critical Reviews in Environmental Science and Technology*, *45*(24), 2693–2727.
13. Kim, J. & Lee, C. (2015). Response of a continuous biomethanation process to transient organic shock loads under controlled and uncontrolled pH conditions. *Water Res.*, *73*:68–77.
14. Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequenc.pdf. *Applied and Environmental Microbiology*, *79*(17), 5112–5120.
15. Lin, H., Peng, W., Zhang, M., Chen, J., Hong, H., & Zhang, Y. (2013). A review on anaerobic membrane bioreactors: Applications, membrane fouling and future perspectives. *Desalination*, *314*, 169–188.
16. Liu, Y., & Whitman, W. B. (2008). Metabolic, phylogenetic, and ecological diversity of the methanogenic archaea. *Annals of the New York Academy of Sciences*, *1125*, 171–189.
17. Mitra, I. N., Sengupta, A. K., Kugelman, I. J., & Creighton, R. (1998). Evaluating composite ion exchangers (CIX) for improved stability of anaerobic biological reactors. *Water Research*, *32*(11), 3267–3280.
18. Mosbæk, F., Kjeldal, H., Mulat, D. G., Albertsen, M., Ward, A. J., Feilberg, A., & Nielsen, J. L. (2016). Identification of syntrophic acetate-oxidizing bacteria in anaerobic digesters. *The ISME Journal*, *2*, 1–14.
19. Nielfa, A., Cano, R., & Fdz-Polanco, M. (2015). Theoretical methane production generated by the co-digestion of organic fraction municipal solid waste and biological sludge. *Biotechnology Reports*, *5*(1), 14–21.

20. Ozgun, H., Dereli, R. K., Ersahin, M. E., Kinaci, C., Spanjers, H., & Van Lier, J. B. (2013). A review of anaerobic membrane bioreactors for municipal wastewater treatment: Integration options, limitations and expectations. *Separation and Purification Technology*, *118*, 89–104.

21. Padmasiri, S. , Zhang, J., Fitch, M., Norddahl, B., Morgenroth, E., & Raskin, L. (2007). Methanogenic population dynamics and performance of an anaerobic membrane bioreactor (AnMBR) treating swine manure under high shear conditions. *Water Research*, *41*:134–144.

22. Park, H., Choo, K.-H., & Lee, C.-H. (1999). Flux Enhancement with Powdered Activated Carbon Addition in the Membrane Anaerobic Bioreactor. *Separation Science and Technology*, *34*(14), 2781–2792.

23. Rebecchi, S., Pinelli, D., Bertin, L., Zama, F., Fava, F., & Frascari, D. (2016). Volatile fatty acids recovery from the effluent of an acidogenic digestion process fed with grape pomace by adsorption on ion exchange resins. *Chemical Engineering Journal*, *306*, 629–639.

24. APHA. (2012). *Standard Methods for the Examination of Water and Wastewater* (22nd ed.). American Public Health Association, American Water Works Association, Water Environment Federation.

25. Schloss, P. D. (2009). Introducing mothur: open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Applied and Environmental Microbiology*, *75*(23), 7537–7541.

26. Senturk, E., Ince, M., & Engin, G. O. (2014). The effect of shock loading on the performance of a thermophilic anaerobic contact reactor at constant organic loading rate. *Journal of Environmental Health Science and Engineering*, *12*(84).

27. Stuckey, D. C. (2012). Recent developments in anaerobic membrane reactors. *Bioresource Technology*, *122*, 137–148.

28. Trzcinski, A. P. (2009). *Anaerobic membrane bioreactor technology for solid waste stabilization*. PhD Dissertation. Imperial College London.

29. Venkiteshwaran, K., Milferstedt, K., Hamelin, J., & Zitomer, D. H. (2016). Anaerobic digester bioaugmentation influences quasi steady state performance and microbial community. *Water Research*, *104*, 128–136.

30. Yoo, R., Kim, J., McCarty, P. L., & Bae, J. (2012). Anaerobic treatment of municipal wastewater with a staged anaerobic fluidized membrane bioreactor (SAF-MBR) system. *Bioresource Technology*, *120*, 133–139.

CHAPTER 5: MIXED MICROBIAL CULTURE POLYHYDROXYALKANOATE PRODUCTION FROM HYDROLYZED MUNICIPAL ORGANIC WASTE

Abstract

Production of the biopolymer, polyhydroxyalkanoate (PHA), from hydrolyzed municipal organic waste was investigated as a strategy for resource recovery from organic waste streams. PHA production using organic waste streams, such as sewage sludge or the organic fraction of municipal solids waste (OFMSW), can be a sustainable alternative for managing organic waste, providing economic opportunity for waste treatment plants while conserving natural resources. PHAs are bacterial polyesters usually produced from volatile fatty acids (VFA) that can be used as biodegradable bioplastics. This study investigated PHA production in three phases: (1) fermentation of the OFMSW to produce a VFA-rich liquid effluent, (2) application of that VFA-rich fermentation liquid to select for PHA accumulating biomass, and (3) accumulation of PHA in the selected biomass using varying concentrations of the fermentation liquid to assess the effects of ammonium-nitrogen concentration on PHA accumulation. Preliminary batch testing to determine optimal operating parameters for the fermentation phase revealed that 5.4% solids content, 37°C, and 3.4 day retention time led to the highest VFA production. Up to 14 g/L VFA production was achieved in lab-scale continuous fermentation of the OFMSW. The liquid fraction of the fermented material was applied using a feast/famine feed strategy to successfully select PHA accumulating biomass. Finally, in the PHA accumulation phase an average maximum yield of 38% PHA/g VSS was achieved using a low ammonium-nitrogen feed mixture. Application of clinoptilolite was determined to be an effective means for reducing ammonium-nitrogen concentration in the fermentation liquid and improving PHA accumulation by up to 29%. Overall, this study demonstrated the feasibility of using a complex organic waste stream, namely municipal organic waste, for mixed microbial culture PHA production, with potential for nutrient recovery, although further optimization of the three-phase process should be investigated to maximize PHA yield and improved overall process efficiency.

Introduction

Global plastic production has increased by more than 20 fold over the last 50 years, with more than 311 billion tons of plastics produced in 2014 (Opsomer & Pennington, 2016). Currently, the majority of plastics are produced from non-renewable crude oil. Around 4 percent

of world oil production is used for plastics production (Gourmelon, 2015). On top of this, plastic materials are often used for only short-term applications, such as plastic bags and packaging, and then disposed of. Only 14% of plastic packaging is collected for recycling while the remaining 86% is either buried in landfills, incinerated, or lost into the natural environment (Opsomer & Pennington, 2016). As a result, plastic waste contributes to significant environmental pollution due to its non-biodegradability. At the same time, with increasing population growth and urbanization, the worldwide production of municipal wastes has been continuously increasing with no signs of significant decrease (Hoornweg & Bhada-Tata, 2012). Among these municipal wastes, the organic fraction of municipal solid waste (OFMSW) is an important component, due to both the amount produced with respect to the total production of municipal waste and its potential to be transformed into valuable products, including biopolymers. Biopolymers as an alternative to petroleum based polymers provide the advantage of conserving fossil fuel resources and reducing CO_2 emissions, making them an important innovation for sustainable development (Bugnicourt, 2014). Due to their biodegradability, biocompatibility, chemical-diversity, and being manufactured from renewable carbon resources, polyhydroxyalkanoates (PHAs) are considered one of the most promising biopolymers as an alternative to petroleum based plastics. Therefore, utilizing municipal organic waste for the production of PHA can provide a dual advantage: converting a waste into a valuable product and avoiding the production, use, and disposal of petroleum-based plastics.

In general, production of PHA utilizing waste streams such as the OFMSW, in combination with mixture microbial cultures (MMC) presents many advantages when compared with current conventional PHA production processes. Presently, industrial processes for PHA production are based on the use of pure microbial cultures of selected strains that then requires use of single, pure substrates for cultivation (Setiadi et al., 2015, Villano et al., 2014). This makes PHA production expensive and uncompetitive with synthetic thermoplastics, due to the costs of culture maintenance, substrate formulation, and both substrate and reactor sterilization (Ivanov et al., 2015). For this reason, use of mixed microbial strains coming from waste has been recognized as a promising alternative to pure culture PHA production, as it does not require maintaining sterile conditions and can be combined with the use of low-cost waste feedstocks (Valentino et al., 2017; Villano et al., 2014). PHA production from MMCs is based on the natural selection of PHA accumulating microorganisms via application of selective pressures to

the community, such as carbon or nutrient limitation. Typically the process is carried out in two steps: selection of PHA accumulating bacteria on the substrate using a feast/famine regime, followed by accumulation of PHA within the selected biomass under nutrient limited conditions (Korkakaki et al., 2016; Serafim, et al., 2008).

Several studies have shown the feasibility of using mixed microbial cultures and various waste feedstocks to achieve PHA enriched biomass. With industrial wastewaters such as paper mill, olive mill, molasses, and candy-bar wastewaters, researchers have been able to achieve 50-80% of PHA in the biomass (Albuquerque et al., 2010; Bengtsson et al., 2008; Jiang et al., 2012; Tamis et al., 2014). However, with more complex waste streams such as municipal wastewater and municipal solid waste, lower PHA yields have been achieved ranging from 9-53% (Basset et al., 2016; Coats et al., 2007; Frison et al., 2014; Korkakaki et al., 2016; Morgan-Sagastume, et al., 2010 & 2015). To date, few studies have investigated the use of OFMSW as a feedstock for PHA production. Korkakaki et al. (2016) used leachate of OFMSW for PHA production and achieved a PHA content of 29%, while Basset et al. (2016) achieved a PHA yield of 10.6% using fermented OFMSW. In general, achieving high PHA content and volumetric productivity with complex waste streams and mixed cultures is still a significant challenge due to issues of high solids and nutrients content which can hinder the selection phase (Serafim, et al., 2008; Tamis et al., 2014).

The objective of this study was to investigate the feasibility of using a complex organic waste stream, specifically non-source-sorted OFMSW referred to as residual organic matter (ROM), as a feedstock for PHA production. The process was investigated in three phase: (1) fermentation of the ROM to produce volatile fatty acids as the substrate for PHA production, (2) selection of PHA accumulating microorganisms via feast/famine, and (3) PHA accumulation in the selected biomass. During the selection phase, comparison of PHA yield using a synthetic substrate as well as the liquid fraction of the fermented ROM with normal and low ammonium-nitrogen concentrations was made in order to evaluate the impact of ammonium-nitrogen levels on PHA accumulation.

Materials and Methods

Substrate and Inoculum

The ROM used in this study was obtained from a mechanical biological treatment (MBT) plant for non-source-sorted municipal waste in the Barcelona metropolitan area. It was collected in 20 liter carboys and stored at 4°C before use. Average total and volatile solids of the ROM were 71.6 ±14.4, and 50.4 ± 9.3 g/L, respectively. Anaerobic digester effluent from the same MBT plant served as inoculum for the fermentation phase of this study. The biomass selection reactor was inoculated with waste activated sludge from a municipal wastewater treatment plant of the Barcelona metropolitan area.

Experimental Set-up

Fermentation Phase

Three phases of operation were investigated in this study: fermentation, biomass selection, and PHA accumulation. Figure 5.1 shows the three reactors used for each phase. A jacketed fermentation reactor with a working volume of 5L, and mechanical stirring (IKA-Werke, RW16 basic) was operated for the production of VFA from ROM. The fermenter was initially loaded with 50% v/v ROM and anaerobic digester effluent as inoculum, and operated at an initial HRT of 2.5 days with the aim of washing out methanogenic microorganisms present in the inoculum. Later operating conditions in the fermentation reactor were determined based on results from an initial fermentation batch test which aimed to optimize VFA production. Optimal temperature for the fermentation reactor was determined to be 37ºC which was maintained via a water bath (Thermo Electron Corporation, HAAKE DC30). Over the course of the study, HRT in the fermenter was increased from 2.5 to 3.5 days. Manual subtractions and additions of material were made daily to maintain the desired HRT.

A preliminary batch fermentation study was conducted to determine optimal conditions for fermentation of ROM to VFA. Effluent collected during start-up of the fermentation reactor served as inoculum for the batch test. Three optimization variables were investigated: solids concentration (3.3, 4.4, 5.6 and 6.1% TS), temperature (33, 35 and 37ºC), and hydraulic retention time (0-5 days). Batch tests were performed in 500 mL serum bottles. To maintain the desired temperature, bottles were submerged in a thermal bath (Thermo Electron Corporation, HAAKE

DC30). The test was carried out for a period of 5 days with regular measurements of VFA concentration.

Figure 5.1. Photos of the three reactors used in this study: (a) continuous fermentation reactor, (b) sequencing-batch biomass selection reactor, and (c) fed-batch PHA accumulation reactor.

Biomass Selection Phase

After fermentation, the effluent (hydrolyzed ROM) was sieved to remove large particles and then filtered via an ultrafiltration membrane (Tami Industries, No. 26110) to achieve separation of the solid and liquid fractions. The liquid fraction of the hydrolyzed ROM served as the substrate for the biomass selection phase. This fermentation liquid was preserved at 4ºC to reduce loss of VFA prior to feeding in the biomass selection phase.

The biomass selection phase consisted of a jacketed sequencing batch reactor (SBR) of 3L. The reactor was operated with mechanical stirring (IKA-Werke, RW16 basic) and temperature was maintained at ambient conditions. This reactor was equipped with 3 peristaltic

pumps connected to programmable timers to automatically control: feeding, effluent withdrawal, and biomass purging. The reactor was also equipped with pH, oxidation-reduction potential (ORP) and dissolved oxygen (DO) probes all connected to a data collection system (Modules Advantech's ADAM) controlled by a program developed in Advantech ADAMView.

The biomass selection SBR was operated in 8 hour cycles under a feast-famine feeding regime. A feast/famine ratio of 0.2 was targeted, consistent with the range of 0.20-0.25 reported in Albuquerque et al. (2010). HRT and SRT were maintained around 7 and 18.5 days respectively. Each cycle in the selection phase SBR consisted of a 2 min feed period, followed by 7.2 hours of reaction time with aeration and mixing, 30 minutes without aeration to allow for any potential denitrification and dissipation of nitrogen gas to occur prior to biomass settling, and finally, a 15 min biomass settling period and 1 min effluent withdrawal period . Some modifications to this cycle were made during the course of the study in response to observed denitrification activity during the biomass settling period.

PHA Accumulation Phase

For the accumulation of PHA, a 1L reactor was set-up and operated in semi-batch mode with regular addition of substrate. Each accumulation test was carried out for a period of 24 hours. At the start of each test, biomass collected from the selection reactor was used as inoculum in the accumulation reactor. 150 mL of selection phase biomass purge was collected, rinsed with deionized water, combined with 600 mL deionized water, and added to the accumulation phase reactor. The purpose for rinsing the biomass was to eliminate the influence of nutrients coming from the selection phase effluent on PHA accumulation. The accumulation phase reactor was operated with continuous mechanical stirring (IKA-Werke, RW16 basic) and temperature was maintained at ambient conditions.

To assess the viability of the ROM fermentation liquid as a feedstock for PHA accumulation, three different substrate mixtures were tested: (1) a primarily synthetic feed mixture, which consisted of 1% v/v fermentation liquid and 1% v/v acetic acid in deionized water, (2) a mixture of 33% v/v fermentation liquid and 1% v/v acetic acid in deionized water, and (3) 100% fermentation liquid. Over the course of each 24 hour test period, automatic feedings of 50 mL substrate were made every four hours to the reactor via a peristaltic pump (Spectra, Pericom-1) and programmer. The four hour feed interval was determined via

observation of the DO profile and time taken for DO in the accumulation reactor to return to endogenous levels after feeding. VFA, total and volatile suspended solids, and PHA concentration in the biomass were measured at the beginning and the end of each 24 hour test to compare the effects of the different substrate mixtures on PHA accumulation.

Ammonium Nitrogen Removal via Clinoptilolite

Application of the natural zeolite, clinoptilolite, as a means for reducing ammonium-nitrogen concentration in the hydrolyzed ROM feedstock, was also investigated. The clinoptilolite used in this study (Zeolite Natural AQUA, 0.5-1 mm) was provided by ZeoCat Soluciones Ecológicas S.L.U. (Barcelona, Spain). Prior to use, the clinoptilolite was rinsed with deionized water and preconditioned into the sodium form by soaking in a 10g/L NaCl solution for 24 hours (Jorgensen & Weatherley, 2003). To determine an appropriate dosage of clinoptilolite, a batch test was conducted applying various concentrations of clinoptilolite (0, 250, 500, 650, 850, 1000 g/L) to 25 mL of filtered fermentation effluent in media bottles, and the change in the liquid's ammonium-nitrogen concentration was measured after 24 hours. Each clinoptilolite dosage was tested in duplicate. After determining an appropriate dosage, the clinoptilolite was added to 1 liter of fermentation liquid for a period of 24 hours to produce enough low nitrogen feed for application in the PHA accumulation phase.

Analytical Methods

All analyses, including total solids (TS), volatile solids (VS), and chemical oxygen demand (COD), were performed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). VFAs were measured using a Shimadzu GC-2010+ gas chromatograph equipped with a capillary column Nukol (0.53 mm ID; 15 m length) and a flame ionization detector (FID). Ammonium-nitrogen concentration (NH_4^+-N) was analyzed with a specific ammonia electrode (ORION 9512). Inorganic cations and anions were measured by ionic chromatography (Metrohm Advanced Compact IC). PHA extraction and quantification was performed via solvent extraction following the method of Basset et al. (2016).

Results and Discussion

Proposed PHA Production System

The initial aim of this research was to show the feasibility of producing PHA from the complex substrate that is non-source-sorted OFMSW (ROM) using a mixed biomass. Figure 5.2 shows a schematic outline of the three-phase process (Reis et al., 2011; Katsou et al., 2015; Frison et al., 2015; Basset et al., 2016) which includes (1) Fermentation of the ROM to produce a fermentation liquid rich in VFA, (2) Selection of PHA accumulating microorganisms using a feast/famine strategy, (3) Accumulation of PHA in the selected biomass. With this process there is also potential for energy production via anaerobic digestion of the solids fraction remaining after fermentation, and nutrient recovery from the liquid fraction prior to use for PHA production.

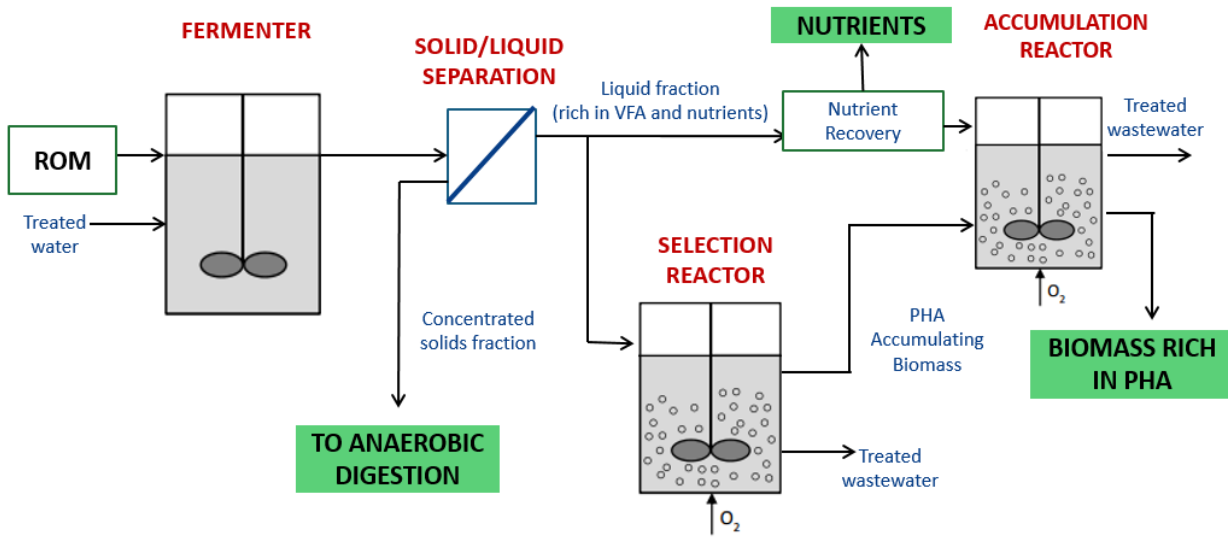


Figure 5.2. Three-phase process for PHA production from residual organic matter (ROM) with potential for nutrient and energy recovery.

Fermentation of ROM

A preliminary batch test was carried out to determine the appropriate operational conditions for achieving maximum VFA production from ROM. The influence of three operational parameters was investigated: solids concentration (from 3.3 to 6.1% TS), temperature (33-37ºC) and retention time (0-5 days). Batch fermentation tests were performed and VFA production results were analyzed using surface response curve methodology to determine

optimal operating parameters for maximum VFA production. Higher VFA production was registered at the highest temperature tested, namely 37ºC. Figure 5.3 shows the response curve resulting from batch fermentation of ROM at 37°C. When comparing the resulting VFA concentrations at various retention times and solids concentrations, it was concluded that maximum VFA production under short-term conditions was achieved at 5.4% TS and 3.4 days of retention time, at 37ºC.

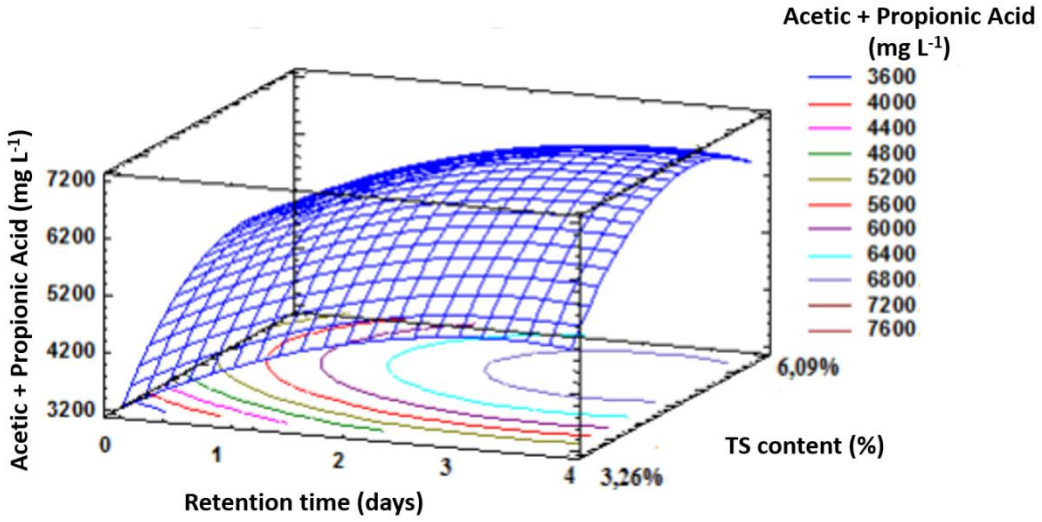


Figure 5.3. Surface response curve from batch test fermentation of residual organic matter (ROM) at 37°C, comparing resulting VFA concentrations at various retention times and total solids concentrations.

Having identified optimal operational parameters in the short-term, a lab-scale continuous fermentation reactor (working temperature 37ºC) was set-up and operated to assess the production of VFA under long-term conditions. Figure 5.4 shows VFA production in the continuous fermenter over a range of HRTs from 2.5 to 3.5 days, where the average TS concentration of the influent ROM was in the range of 45.7 ± 10.2 g L^{-1}. In Figure 5.4, it can be observed that VFA concentrations increased with increasing HRT, although it was also dependent on the feed substrate (see initial VFA concentration). Average VFA concentrations at 2.5, 3.0, and 3.5 day HRT were 8,880 ±765; 9,359 ±1,506; 11,886 ±1,360 mg L^{-1}, respectively. Moreover, longer retention time resulted in a greater proportion of acetic and propionic acid within the total mixture of VFAs produced. Acetic acid represented an average percentage of 35.2, 37.5 and 45.3% of the total VFA generated in the fermentation reactor for HRT 2.5, 3.0 and 3.5 days, respectively. However, it should be highlighted that the ROM fed in the last period

(HRT 3.5 days) contained more VFA than that used in the previous periods. Considering the VS mass balance, effluent VS represented approximately 90% of VS fed to the reactor. The 10% difference could be related to VS degradation, accumulation of solids in the bottom of the fermentation reactor and/or biogas production.

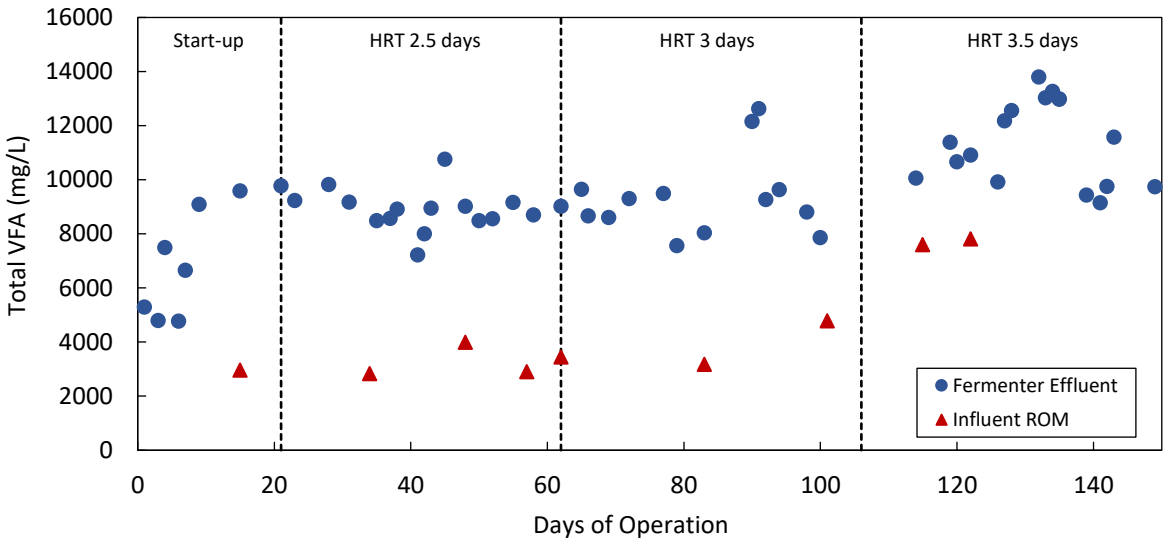


Figure 5.4. Concentration of total VFA in influent and effluent of the lab-scale continuous fermenter treating the residual organic matter (ROM) over time with increasing hydraulic retention time (HRT).

After fermentation of the ROM, the liquid and solid fractions of the fermentation liquid were separated via membrane filtration. The resulting liquid fraction, rich in VFA, was applied as substrate in the biomass selection phase of the process. Table 5.1 describes pH, VFA content and NH_4^+-N concentration of the ROM before fermentation, the resulting fermentation effluent before filtration, and the final fermentation liquid fraction after filtration. From Table 5.1 it can be seen that there was a minor loss (8%) of VFA during the filtration process.

Table 5.1. Characteristics of residual organic matter (ROM), fermentation-phase effluent, and filtered fermentation liquid.

Parameter	Units	Residual Organic Matter (ROM)	Fermentation Effluent (before filtration)	Liquid Fraction of Fermentation Effluent
pH	-	6.3 ±0.3	6.0 ±0.4	6.2 ±1.4
Total VFA	mg/L	4,388 ±1,982	9,492 ±1,931	8,700 ±356
NH_4^+-N	mg/L	1,794 ±631	2,087 ±779	2,079±725

Selection of PHA Accumulating Biomass

Selection of PHA-accumulating microorganisms from activated sludge inoculum was accomplished via 8 hour cycles of feast/famine in an SBR fed with VFA. An average feast/famine time ratio ranging from 0.15 to 0.21, was achieved, which falls near the range of 0.20-0.25 reported by Albuquerque et al. (2010). Total VFA concentration in the clarified effluent was always below 30 mg VFA L^{-1}, resulting in a VFA removal efficiency above 99%. Figure 5.5 shows the evolution of TSS and VSS in the mixed liquor and the percentage of PHA in the purged biomass. Several operating strategies were applied in the selection reactor over the course of this study, which can be divided into 4 different operating periods as shown in Figure 5.5. During start-up, Period 1 (0-15 days), the SBR was fed with diluted fermentation liquid (50% v/v) in deionized waster spiked with acetic acid in order to obtain 6 g VFA L^{-1} in the feed. During this period, HRT and SRT were set at 7.5 and 17 days, respectively and average VSS and TSS stabilized to around 2.5 and 2.7 g/L, respectively. PHA content in the effluent biomass was in the range of 1.5-2.5% (on VSS basis).

During the start-up period, aeration was applied to the reactor for only 7.2 hours of the SBR cycle, with the 30 minutes before biomass settling and effluent withdrawal dedicated to mixing without aeration to promote denitrification. However, this resulted in problems during the sludge setting period, as the formation of nitrogen gas led to sludge flotation. Therefore, in a second period of operation, (days 16-63), a 30 min anoxic stage was added directly after feeding in every SBR cycle in order to avoid denitrification during the settling step. This strategy led to a decrease in the PHA content of the purged biomass (1.2-1.7% on VSS basis), likely due to a reduction of available VFA at the start of the feast phase under aerobic conditions (part of the VFAs were consumed for denitrification) and to the proliferation of heterotrophic denitrifying biomass and autotrophic nitrifying biomass without PHA storing capacity. For this reason, in a third period of operation (days 64-105), the anoxic period after feeding was discontinued, and as a result the PHA content of purged biomass increased. Finally, from day 105 on (Period 4), undiluted fermentation liquid was fed to the SBR, HRT and SRT during this period were set at 6 and 20 days, respectively, and an increase in both the volatile suspended solids and the PHA content of purged biomass (13.8-17.6% on VSS basis) was observed.

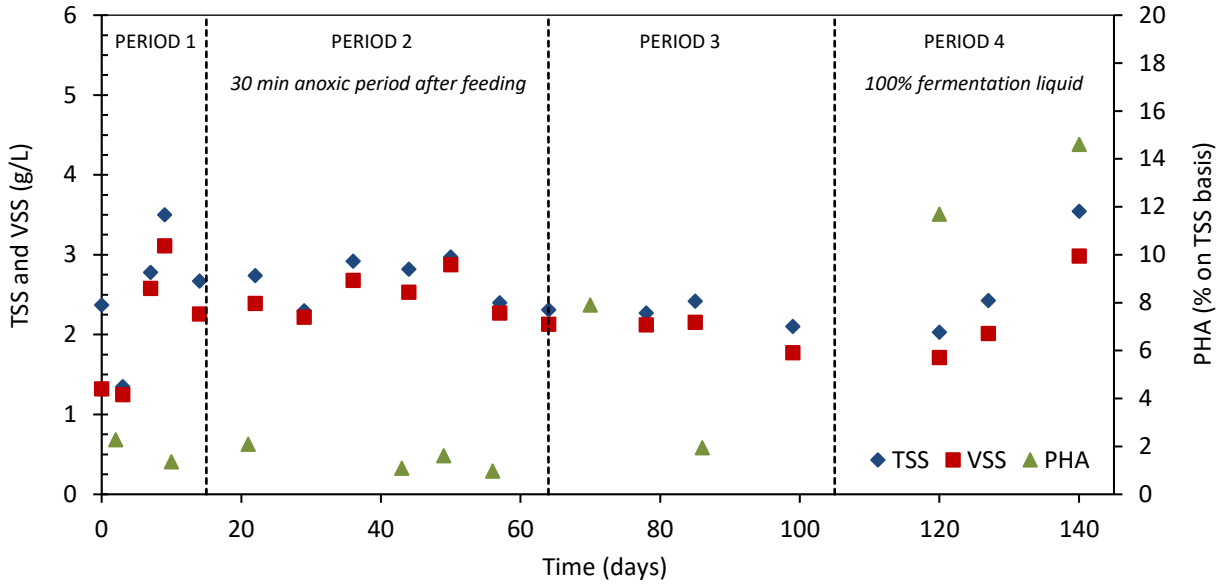


Figure 5.5. Biomass selection phase total suspended solids (TSS), volatile suspended solids (VSS), and percent PHA per gram VSS obtained over time.

Table 5.2 shows the operating conditions during Period 4, when undiluted fermentation liquid was fed to the reactor. It is important to highlight that during this stage elevated pH values (in the range of 9.4) were observed which promoted high free ammonia concentrations and consequent nitrification inhibition. Total ammonia nitrogen was in the range of 1.1-1.3 g NH_4^+-N L^{-1}, so NH_3 stripping also occurred. In order to avoid NH_3 stripping in the future, removal/recovery of ammonia from the hydrolyzed ROM prior to its application for biomass selection (and PHA accumulation) should be applied. One method for ammonia recovery, ion-exchange via clinoptilolite, was investigated in the PHA accumulation phase of this study and is discussed later.

Figure 5.6 shows a representative dissolved oxygen (DO) profile in the selection reactor over time, for 4 consecutive SBR cycles (HRT of 6 days). This profile reflects the feast and famine periods achieved in the reactor, with low DO reflecting the period of VFA consumption (feast), and increasing/high levels of DO reflecting the period of famine. During the famine period, bacteria that are able to store organic carbon as PHA have an advantage, as they can use their stored PHA for energy (Lee, 1996; Van Loosdrecht et al., 1997). Thus, with multiple cycles of feast and famine (in this case, with a feast to famine time ratio of 0.15) selection of PHA accumulating microorganism was achieved. Biomass samples were collected after feast periods

to determine the PHA concentration. The maximum concentration achieved was 17.6% of PHA (on VSS basis), confirming successful selection of PHA accumulators in the biomass.

Table 5.2. Operating conditions in the biomass selection reactor fed 100% fermentation liquid (Period 4).

Parameter	Average Value	Range Value	Units
Cycle duration	8	-	h
Feeding (with mixing)	2	-	min
Aeration + mixing	432	-	min
Mixing (without aeration)	30	-	min
Settling	15	-	min
Effluent withdrawal	1	-	min
OLR	1.29	0.90-1.67	g VFA (L day)$^{-1}$
% VFA removal	>99	-	%
HRT	6	-	days
SRT	20	-	days
TSS	3.02	2.03-3.54	g SS L^{-1}
VSS	2.47	1.72-2.99	g VSS L^{-1}
Feast/Famine time ratio	0.15	0.14-0.15	-
% PHA in the purged biomass	15.7	13.8-17.6	% (on VSS basis)

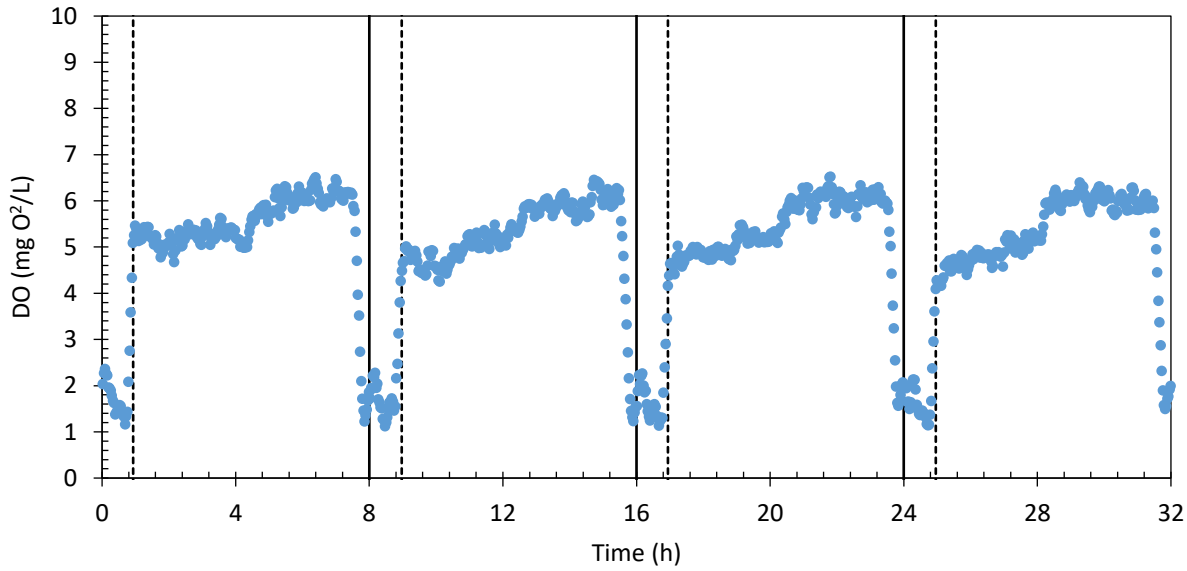


Figure 5.6. Dissolved oxygen profile in biomass selection reactor, over repeating 8 hour reaction cycles, reflecting the feast/famine feed strategy during operation with 100% fermentation liquid (Period 4). (End of SBR cycle: ——— ; End of Feast period in each SBR cycle: - - -).

PHA Accumulation and Ammonium Nitrogen Recovery via Clinoptilolite

In the final phase of the process, PHA accumulation within selected biomass was investigated. PHA accumulation in select microorganisms occurs when they are subjected to stress conditions such as limitation of a nutrient, electron donor or acceptor. In this case, nutrient limitation was the chosen strategy for PHA accumulation, and the impact of ammonium nitrogen concentration on PHA accumulation was investigated. Biomass collected from the selection reactor (mainly during Period 2 and 3) was used to inoculate each batch test conducted in the PHA accumulation reactor. Prior to seeding the reactor, the biomass was rinsed to remove nutrients that were present in the liquid effluent of the selection reactor. Three concentrations of filtered fermentation liquid (1%, 33%, and 100%) were applied as substrate in the accumulation reactor to achieve a comparison of low, medium, and high ammonium-nitrogen addition. In the 1% and 33% conditions, acetic acid was added to the dilution so that the level of VFA in all three substrate mixtures was similar (approx. 6 g/L). The majority of the fermentation liquid that was used for PHA accumulation testing was collected during Period 2 of the fermentation phase, when average acetic acid represented approximately 34% of total VFA. The fermentation liquid was stored in the refrigerator prior to use in an attempt to reduce the loss of VFA, however, some loss of VFA did occur. The three substrate mixtures are characterized in Table 5.3.

Table 5.3. Characterization of PHA accumulation substrate mixtures.

Fermentation Liquid Concentration	1%	33%	100%	
Total VFA	6050 ±705	5839 ±1345	5722 ±1512	mg/L
Acetic	99.2	64.6	32.0	% of Total
Propionic	0.2	14.8	39.1	
Isobutyric	0.0	5.3	11.4	
Butyric	0.2	5.3	13.2	
NH_4^+-N	6.9 ±4	725.3*	2,198 ±716	mg/L
N/COD	0.46	49.8	114.3	mg/g
pH	5.7 ±0.3	5.7 ±0.5	6.2 ±1.4	-

*Calculated based on NH_4^+-N concentration in 100% fermentation liquid

A series of accumulation batch tests was performed using the different substrate mixtures. Results are shown in Table 5.4. The maximum PHA yield achieved was 45% on VSS basis (37% on TSS basis) using the 1% fermentation liquid feed mixture. Overall, average PHA yield was highest in the 1% feed mixture, and decreased with increasing concentration of fermentation liquid. Average PHA yield in 1, 33, and 100% fermentation liquid were 38, 27, and 19% per g VSS, respectively.

Table 5.4. PHA accumulation batch test parameters and results, comparing different concentrations of fermentation liquid in the substrate (1%, 33% and 100%).

Fermentation Liquid Concentration		1%	33%	100%	
OLR		1.9 ±0.35	1.86 ±0.56	1.86 ±0.58	kg VFA/m^3 d^{-1}
Initial F:M		0.63 ±0.18	0.98 ±0.11	0.93 ±0.16	g VFA/g TSS
VFA removal		58 ±25	50	44 ±13.24	%
TSS	*Initial*	0.74 ±0.29	0.46 ±0.10	0.49 ±0.09	g/L
	Final	1.29 ±0.62	1.15 ±0.13	1.54 ±0.09	
VSS	*Initial*	0.62 ±0.30	0.42 ±0.07	0.47 ±0.09	g/L
	Final	1.18 ±0.68	1.07 ±0.16	1.42 ±0.08	
PHA	*Initial*	2.3 ± 0.2	7.5 ± 9.0	6.2 ± 3.9	% VSS
	Final	**37.5 ±6.3**	**27.1 ±5.8**	**18.8 ± 5.8**	

In general, PHA yields obtained in this study were near the range of previously reported PHA accumulation values. Serafim et al. (2008) reported a range of 20-54% PHA per g biomass for PHA accumulation using aerobic dynamic feeding strategies with mixed cultures and complex substrates. In this study, the lower PHA yield observed with increasing concentration of fermentation liquid is likely due to the increase of nutrients. Previous research has found that high levels of nutrients can cause inhibition of PHA accumulation. Korkakaki et al. (2016), who was able to achieve 29% PHA using leachate of OFMSW as the substrate, presented experimental data that confirmed that specific substrate uptake rates were significantly reduced when OFMSW leachate was used as the substrate as compared to an equivalent artificial VFA mixture. Testing the different possible inhibitors, such as salt, ammonium or VFA concentration suggested that the main inhibition most likely was caused by the high ammonium concentration of the OFMSW leachate. Optimal N/COD of 2-15 mg g^{-1} was reported by Valentino et al.

(2015). In this study, the 33% and 100% fermentation liquid condition had N/COD ratios of 49.8 and 114.3 mg g^{-1} respectively, well beyond the suggested optimal range. Therefore, inhibition of PHA accumulation due to high ammonium concentrations is highly likely, and in order to improve PHA production from ROM, a means for removing and ideally recovering ammonium-nitrogen from the fermentation liquid prior to use is recommended.

To that end, the use of clinoptilolite was investigated as a way for removing ammonium-nitrogen from the fermentation liquid. Clinoptilolite is a natural zeolite with a high cation exchange capacity and affinity for ammonium (Hedstrom, 2001). Therefore, it was expected that adding clinoptilolite to the fermentation liquid would reduce the concentration of ammonium-nitrogen providing a substrate with high VFA and low nitrogen, ideal for PHA accumulation. In this study, a batch test was conducted in which various concentrations of clinoptilolite (0, 250, 500, 650, 850, 1000 g/L) were added to fermentation liquid and the change in the liquid's ammonium-nitrogen concentration was measured after 24 hours. Figure 5.7 shows the resulting levels of ammonium-nitrogen in the fermentation liquid after 24 hours of exposure to different concentrations of clinoptilolite. A 70% reduction in ammonium-nitrogen (from 2097 to 595 mg/L) was seen with the addition of just 250 g/L of clinoptilolite. Removal of ammonium-nitrogen increased with increasing concentration of clinoptilolite. The highest removal, up to 93%, was achieved with the addition of 1000 g/L which reduced the liquid ammonium-nitrogen concentration to as low as 144 mg/L.

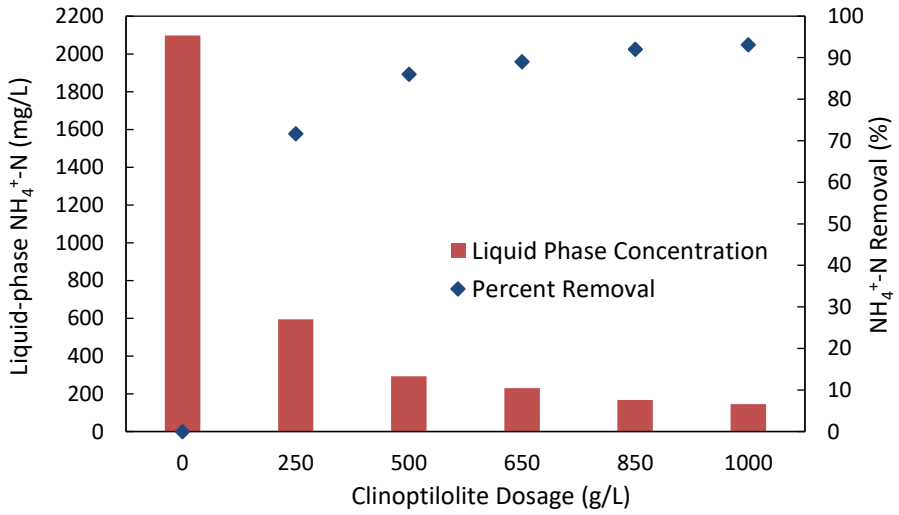


Figure 5.7. Percent removal and equilibrium liquid-phase concentration of ammonium-nitrogen after 24 hours of contact time with various dosages of clinoptilolite (0, 250, 500, 650, 850, 1000 g/L).

Seeing that clinoptilolite could successfully reduce ammonium-nitrogen concentrations in the hydrolyzed ROM, this technique was used to prepare substrate for the 1 L PHA accumulation phase. 500 g/L of clinoptilolite was used to reduced ammonium nitrogen concentrations in the hydrolyzed ROM from 1372 to 353 mg/L achieving a N/COD of 11.85 mg/g. Results from this test are shown in Table 5.5, where it can be seen that reducing the N/COD ratio resulted in higher PHA yield. Hydrolyzed ROM treated with clinoptilolite resulted in 23% PHA per gram of VSS compared to 17% in the case of the untreated, high-nitrogen hydrolyzed ROM. The synthetic feed (1% fermentation liquid), which had the lowest level of ammonium-nitrogen, resulted in the greatest production of PHA, 26% per gram VSS

Table 5.5. Conditions and results for PHA accumulation using hydrolyzed ROM as the substrate with and without removal of NH_4^+-N via clinoptilolite and compared to a synthetic VFA feed mixture.

Condition	Feed NH_4^+-N (mg/L)	Feed VFA (g COD/L)	Feed N/COD (mg/g)	Initial F:M (g COD/g VSS)	PHA (% per g VSS)
Synthetic feed	6.9	18.44	0.37	1.67	25.63
Feed Treated w/ Clinoptilolite	352.8	19.78	11.85	1.69	21.76
Untreated Feed	1372.8	18.71	73.36	1.69	16.83

Overall, PHA accumulation using the liquid fraction of fermented ROM was most successful under conditions of low ammonium-nitrogen concentration, achieving an average maximum PHA yield of 38% on VSS (33% on TSS basis). Due to the high concentration of ammonium-nitrogen contained in the hydrolyzed ROM, increasing the concentration of fermentation liquid in the feed stream increased nitrogen concentrations beyond those optimal for PHA accumulation, and a reduction in PHA yield was observed. Application of clinoptilolite proved to be an effect method for removing ammonium-nitrogen from the fermentation liquid and increasing subsequent PHA accumulation. Further optimization of the PHA accumulation phase parameters, including F:M ratio and frequency of feeding, should be done to further improve PHA yield.

Conclusions

Biopolymer production from organic waste may be a sustainable option for managing the organic fraction of municipal solid waste (OFMSW), providing economic opportunity for treatment plants and an alternative to petroleum based plastics. In this study, the feasibility of PHA production from residual organic matter (ROM) was investigated and verified in a three-phase mixed microbial culture PHA production process. The production of VFA from fermentation of ROM was determined to be optimal under the following conditions: 5.4% solids, 37ºC, and 3.4 day HRT. In the biomass selection phase, feeding the liquid fraction of fermented ROM at a feast/famine ratio of 0.15, SRT of 20 days, and HRT of 6 days, resulted in enrichment of PHA-accumulating biomass with up to 17.6% PHA/g VSS. Finally, biomass from the selection phase was inoculated in a PHA accumulation reactor where a maximum PHA content of 38% on VSS basis (33% on TSS basis) was obtained using a low-nitrogen feed mixture. Using the pure liquid fraction of fermented ROM as substrate in the PHA accumulation phase resulted in reduced PHA production which was attributed to inhibition from high ammonium-nitrogen concentrations. Treating the fermentation liquid with 500 g/L clinoptilolite reduced ammonium-nitrogen concentrations by 74%. Use of this feed in the PHA accumulation phase resulted in 29% greater PHA production compared to use of the untreated fermentation liquid. Overall, this study demonstrates the feasibility of using ROM as a substrate for PHA production, and presents addition of clinoptilolite as a viable method for reducing ammonium-nitrogen concentration and improving PHA yields. Further optimization of the three-phase PHA production system should be investigated, including application of ammonium removal via clinoptilolite prior to the biomass selection phase, in order to maximize PHA production and improve process efficiency.

References

1. Albuquerque, M. G. E., Torres, C. A. V, & Reis, M. A. M. (2010). Polyhydroxyalkanoate (PHA) production by a mixed microbial culture using sugar molasses: Effect of the influent substrate concentration on culture selection. *Water Research*, *44*(11), 3419–3433.
2. Basset, N., Katsou, E., Frison, N., Malamis, S., Dosta, J., & Fatone, F. (2016). Integrating the selection of PHA storing biomass and nitrogen removal via nitrite in the main wastewater treatment line. *Bioresource Technology*, *200*, 820–829.
3. Beccari, M., Bertin, L., Dionisi, D., Fava, F., Lampis, S., Majone, M., Villano, M. (2009). Exploiting olive oil mill effluents as a renewable resource for production of biodegradable polymers through a combined anaerobic-aerobic process. *Journal of Chemical Technology and Biotechnology*, *84*(6), 901–908.
4. Bengtsson, S., Werker, A., & Welander, T. (2008). Production of polyhydroxyalkanoates by glycogen accumulating organisms treating a paper mill wastewater. *Water Science and Technology*, *58*(2), 323–330.
5. Bugnicourt, E., Cinelli, P., Lazzeri, A., & Alvarez, V. (2014). Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging. *Express Polymer Letters*, *8*(11), 791–808.
6. Coats, E. R., Loge, F. J., Wolcott, M. P., Englund, K., & McDonald, A. G. (2007). Synthesis of Polyhydroxyalkanoates in Municipal Wastewater Treatment. *Water Environment Research*, *79*(12), 2396–2403.
7. Coats, E. R., VandeVoort, K. E., Darby, J. L., & Loge, F. J. (2011). Toward Polyhydroxyalkanoate Production Concurrent with Municipal Wastewater Treatment in a Sequencing Batch Reactor System. *Journal of Environmental Engineering*, *137*(1), 46.
8. Ellen MacArthur Foundation. (2016). *The New Plastics Economy: Rethinking the future of plastics*. *Ellen MacArthur Foundation*.
9. Frison, N., Katsou, E., Malamis, S., Oehmen, A., & Fatone, F. (2015). Development of a Novel Process Integrating the Treatment of Sludge Reject Water and the Production of Polyhydroxyalkanoates (PHAs). *Environmental Science and Technology*, *49*(18), 10877–10885.
10. Gourmelon, G., Mármol, Z., Páez, G., Rincón, M., Araujo, K., & Aiello, C. (2015). Global Plastic Production Rises, Recycling Lags. *Revista Tcnocientifica URU*, (April 2016), 1–7.
11. Hoornweg, D., & Bhada-Tata, P. (2013). What a waste: A Global Review of Solid Waste Management, 116.
12. Ivanov, V., Stabnikov, V., Ahmed, Z., Dobrenko, S., & Saliuk, A. (2014). Production and applications of crude polyhydroxyalkanoate-containing bioplastic from the organic fraction of municipal solid waste. *International Journal of Environmental Science and Technology*, *12*(2), 725–738.
13. Jiang, Y., Marang, L., Tamis, J., van Loosdrecht, M. C. M., Dijkman, H., & Kleerebezem, R. (2012). Waste to resource: Converting paper mill wastewater to bioplastic. *Water Research*, *46*(17), 5517–5530.
14. T.C. Jorgensen, & L.R. Weatherley. (2003). Ammonia removal from wastewater by ion exchange in the presence of organic contaminants. *Water Research*, 37, 1723-1728
15. Katsou, E., Malamis, S., Frison, N., & Fatone, F. (2015). Coupling the treatment of low strength anaerobic effluent with fermented biowaste for nutrient removal via nitrite. *Journal of Environmental Management*, *149*, 108–117.
16. Korkakaki, E., Mulders, M., Veeken, A., Rozendal, R., van Loosdrecht, M. C. M., & Kleerebezem, R. (2016). PHA production from the organic fraction of municipal solid waste (OFMSW): Overcoming the inhibitory matrix. *Water Research*.
17. Lanham, A. B., Ricardo, A. R., Albuquerque, M. G. E., Pardelha, F., Carvalheira, M., Coma, M., Reis, M. A. M. (2013). Determination of the extraction kinetics for the quantification of polyhydroxyalkanoate monomers in mixed microbial systems. *Process Biochemistry*, *48*(11), 1626–1634.
18. Lee, S. Y. (1996). Bacterial Polyb ydroxyalkanoates. *Biotechnology and Bioengineering*, *49*, 1–14.

19. Morgan-Sagastume, F., Hjort, M., Cirne, D., F., Lacroix, S., Gaval, G., Werker, A. (2015). Integrated production of polyhydroxyalkanoates (PHAs) with municipal wastewater and sludge treatment at pilot scale. *Bioresource Technology*, *181*, 78–89.

20. Morgan-Sagastume, F., Karlsson, A., Johansson, P., Pratt, S., Boon, N., Lant, P., & Werker, A. (2010). Production of polyhydroxyalkanoates in open, mixed cultures from a waste sludge stream containing high levels of soluble organics, nitrogen and phosphorus. *Water Research*, *44*(18), 5196–5211

21. Opsomer, R., & Pennington, J. (2016). What Are the Drawbacks of Today's Plastics Economy. Retrieved from www.weforum.org

22. Reis, M., Albuquerque, M., Universidade, F. C. T., & Lisboa, N. De. (2011). *6 . 51 Mixed Culture Processes for Polyhydroxyalkanoate Production from Agro-Industrial Surplus / Wastes as Feedstocks*. *Comprehensive Biotechnology* (Second Edition, Vol. 6). Elsevier B.V.

23. Rice, E. W., Baird, R. B., Eaton, A. D., & Clesceri, L. S. (2012). *Standard Methods for the Examination of Water and* (22nd ed.). American Public Health Association, American Water Works Association, Water Environment Federation.

24. Serafim, L. S., Lemos, P. C., Albuquerque, M. G. E., & Reis, M. A. M. (2008). Strategies for PHA production by mixed cultures and renewable waste materials. *Applied Microbiology and Biotechnology*, *81*(4), 615–628.

25. Setiadi, T., Trianto, A., Aznury, M., & Pancoro, A. (2015). Production of polyhydroxyalkanoate (PHA) by ralstonia eutropha JMP 134 with volatile fatty acids from palm oil mill effluent as precursors. *Water Science and Technology*, *72*(11), 1889–1895.

26. Tamis, J., Lužkov, K., Jiang, Y., Loosdrecht, M. C. M. van, & Kleerebezem, R. (2014). Enrichment of Plasticicumulans acidivorans at pilot-scale for PHA production on industrial wastewater. *Journal of Biotechnology*, *192*(Part A), 161–169.

27. Valentino, F., Karabegovic, L., Majone, M., Morgan-Sagastume, F., & Werker, A. (2015). Polyhydroxyalkanoate (PHA) storage within a mixed-culture biomass with simultaneous growth as a function of accumulation substrate nitrogen and phosphorus levels. *Water Research*, *77*, 49–63.

28. Van Loosdrecht, M. C. M., Pot, M. A., & Heijnen, J. J. (1997). Importance of bacterial storage polymers in bioprocesses. *Water Science and Technology*.

29. Villano, M., Valentino, F., Barbetta, A., Martino, L., Scandola, M., & Majone, M. (2014). Polyhydroxyalkanoates production with mixed microbial cultures: From culture selection to polymer recovery in a high-rate continuous process. *New Biotechnology*, *31*(4), 289–296.

CHAPTER 6: COMPARATIVE ENVIRONMENTAL IMPACT ASSESSMENT OF SEWAGE SLUDGE TREATMENT VIA ANMBR AND PHA PRODUCTION FOR RESOURCE RECOVERY

Abstract

Resource recovery from sewage sludge is a promising strategy for improving the economic and environmental sustainability of wastewater treatment. This study applied life cycle assessment (LCA) methodology to evaluate and compare the environmental impacts associated with the two resource recovery options, i.e. methane recovery via AnMBR treatment, and bio-polymer recovery via MMC PHA production, considering primary sewage sludge as the substrate. Overall, the AnMBR process was determined to be the more environmentally sustainable option achieving a reduced environmental impact in 6 out of the 10 impact categories considered. Energy consumption was determined to be the largest contributor to overall environmental impact for both processes. However, in the case of AnMBR treatment, it was estimated that more than enough energy could be recovered as methane to offset energy requirements and achieve a positive energy balance. In the case of PHA production, the high energy requirements for aeration negatively impacted the global warming potential (GWP) of the PHA process, although it performed better in the impact categories of fossil fuel depletion and ecotoxicity compared to the AnMBR process. Uncertainty and sensitivity analysis suggested that, under optimized conditions, it may be possible to achieve a net negative GWP for PHA production from primary sludge. In addition, an initial economic assessment that included only operating input costs and potential revenue from recovered methane and PHA products suggested that the relatively high selling price of PHA could more than offset the operating input costs for its production, potentially leading to greater economic benefits compared to the AnMBR process. In the end, a combination of the two technologies may be an advantageous option for improving the environmental and economic sustainability of wastewater treatment. However, a more detailed techno-economic analysis, including consideration of capital costs and PHA extraction is needed. In addition, LCA predictions should be validated with large-scale, long-term demonstration of the two technologies.

Introduction

Wastewater treatment is essential for protecting human health and maintaining a healthy environment. However, current conventional wastewater treatment, which focuses primarily on aerobic conversion of organic pollutants to carbon dioxide, requires significant energy input making it costly and environmentally unsustainable. With increasing economic development, population growth, aging infrastructure, and stricter regulations, the energy and material demands of wastewater treatment are only expected to increase (EPA, 2006; Mo & Zhang, 2013). Meanwhile, the carbon content of wastewater represents a potential renewable resource for energy and materials production that could be leveraged to of offset the cost and resource demands of wastewater treatment. Thus, shifting the current the paradigm from pollutant removal to resource recovery is a promising strategy for improving the economic and environmental sustainability of wastewater treatment processes.

This study investigated the environmental impacts of two emerging technologies for resource recovery from sewage sludge, namely methane recovery via anaerobic membrane bioreactor (AnMBR) treatment, and bioplastic recovery via mixed microbial culture (MMC) polyhydroxyalkanoate (PHA) production. Currently, a common method for treating sewage sludge is through anaerobic digestion, in which the organic material contained in sewage sludge is consumed by a consortium of microorganisms and converted into a methane-rich biogas that can be utilized as an alternative to natural gas. Compared to conventional anaerobic treatment, AnMBRs, which combined anaerobic digestion with membrane filtration, offer the advantage of increased methane production, reduced waste sludge production, improved effluent quality, and a smaller footprint (Chang, 2014). On the other hand, directing sewage sludge to MMC PHA production rather than anaerobic treatment may offer greater economic opportunity as the price of PHA is substantially higher than that of methane (Korkakaki et al., 2016). In the case of MMC PHA production, sewage sludge is fermented and filtered to produce a volatile fatty acid (VFA) rich liquid effluent that can be utilized as the feedstock for PHA accumulating microorganism. The recovered PHA polymer can then be used as a biodegradable alternative to petroleum based plastics. While both AnMBR treatment and MMC PHA production offer the opportunity for resource recovery from sewage sludge, both also require significant energy and material input which can reduce the environmental sustainability of either process. Therefore, in order to

evaluate the two processes as potential options for resource recovery from sewage sludge, the environmental impacts of each process should be quantified and compared.

To that end, the objective of this study was to utilize life cycle assessment (LCA) methodology to quantify and compare the potential environmental impacts of primary sludge treatment via AnMBR and MMC PHA production. A preliminary economic analysis was also conducted considering only operational costs and offsets from methane recovery and PHA production. The two processes were modelled based on mass and energy balance calculations using theoretical and empirical data collected from scientific literature. Monte Carlo simulation was applied to evaluate the uncertainty of model predictions and sensitivity analysis was conducted to determine which process parameters have the greatest influence on global warming potential of the two processes.

Methods

Process configuration

The two treatment processes, AnMBR and MMC PHA production, shown in Figure 6.1, were modelled based on energy and mass flows through each unit process using theoretical or empirical data collected from scientific literature. Table 6.1 summarizes the process parameters used to model the two treatment options. A baseline condition for each of the two processes was established using the average or most likely value for all input parameters as described in Table 6.1. Process parameters that were varied for uncertainty analysis include a description of the assumed probability distribution and range values. The substrate for both treatment options was primary sewage sludge as characterized in Table 6.1. These values were based on the studies presented in Chapters 3 and 4 of this dissertation as well as the values presented in Meabe et al. (2013). An influent flow rate of 500,000 liters of primary sludge per day was assumed.

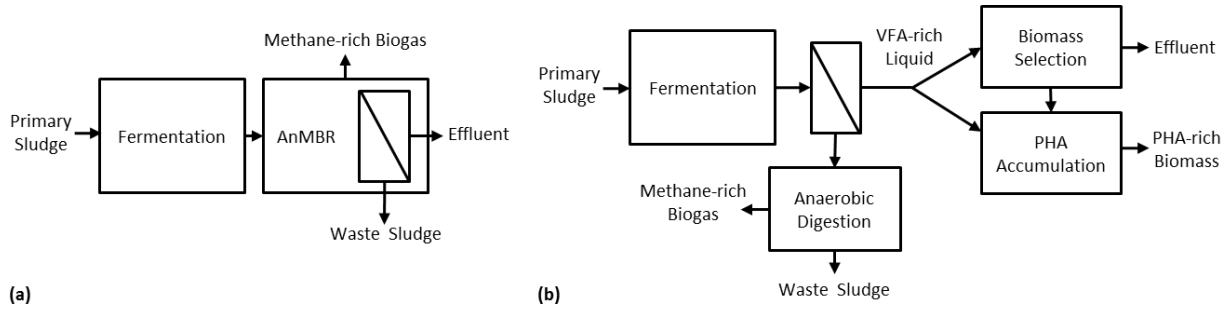


Figure 6.1. Schematic representation of the (a) AnMBR and (b) PHA production process that were modeled and evaluated in this study.

Table 6.1. Process parameters used to model the AnMBR and PHA production processes.

Unit Process	Parameter	Baseline Value	Units	Probability Distribution	Range Values*	References
Primary Sludge	TCOD	36	g/L	Normal	36.0(4.9)	15, 16
	SCOD	6	g/L	Normal	5.9(0.9)	15, 16
	TS	28	g/L	Normal	27.7(4.5)	15, 16
	VS	21	g/L	Normal	21.4(3.5)	15, 16
Fermentation	HRT	3	days			16
	SRT	3	days			16
	Temperature	37	°C			
	Acidification Rate	15	% TCOD	Uniform	15-60	17, 32
	Energy for Heating	0.02	kWh/L			
	Energy for Mixing	0.0065	kW/m^3	Uniform	0.005-0.008	26
Membrane Filtration	Energy Input	2.9	kWh/m^3	Triangular	0.03,2.9,16.52	14
	Flux	12	$L/m^2\ h^{-1}$	Triangular	5,12,20	14, 24
	Membrane Lifetime	10	years	Triangular	5,10,15	24
	NaOCl	2.2	$L\ yr^{-1}\ m^3\ d^{-1}$			24
	Citric Acid	0.6	$L\ yr^{-1}\ m^3\ d^{-2}$			24
AnMBR	HRT	10	days	Uniform	1-12	20
	SRT	70	days	Uniform	30-100	4
	Temperature	37	°C			
	COD Removal Rate	70	%	Uniform	50-90	3, 8, 15
	Methane Yield	0.27	$L/g\ COD_{removed}$	Triangular	0.19,0.27,0.35	3, 15, 16
	Methane in Biogas	67.5	%	Uniform	65-70	26
	Energy for Mixing	0.0065	kW/m^3	Uniform	0.005-0.008	26
PHA Culture Selection	HRT	1	day			9
	SRT	10	days			9
	Biomass Yield	0.3	$g\ COD_{biomass}/g\ COD_{VFA}$			9
	Conversion Factor	1.42	g COD/g VSS			25, 26
	Biomass Concentration	4	g CDW/L			9
	BOD Conversion Factor	0.6	g BOD/g COD			26
	Aeration Required	1.3	$kg\ O_2/kg\ BOD$	Uniform	1-1.5	9
	OTE	15	%	Uniform	10-20	9
	Aeration Efficiency	2.5	$kg\ O_2/kWh$	Uniform	1-3.5	9, 21
PHA Accumulation	HRT	1	day			
	SRT	1	day			
	F:M	9	g COD/g COD	Uniform	3.5-14.5	32, 34
	Biomass Yield	0.15	$g\ VSS/\ g\ COD_{removed}$			19
	Storage Yield	0.6	$g\ COD\ PHA/g\ COD_{VFA}$	Uniform	0.3-0.9	19, 22, 25, 32
	PHA Conversion Factor	1.7	g COD/g PHA			25
	Aeration Required	1.3	$kg\ O_2/kg\ BOD$	Uniform	1-1.5	9
	OTE	15	%	Uniform	10-20	9
	Aeration Efficiency	2.5	$kg\ O_2/kWh$	Uniform	1-3.5	9, 21
Anaerobic Digestion	HRT	25	days			21, 26
	SRT	25	days			
	Temperature	37	°C			
	COD Removal Rate	50	%	Uniform	40-60	
	Methane Yield (35C)	0.27	$L/g\ COD_{removed}$	Triangular	0.19,0.27,0.35	3, 15, 16
	Methane in Biogas	67.5	%	Uniform	65-70	26
	Biomass Yield	0.05	$g\ VSS/g\ COD_{removed}$			
	Energy for Mixing	0.0065	kW/m^3	Uniform	0.005-0.008	26
Biogas Utilization	CHP Efficiency	70	%			27
Sludge Dewatering	Solids Capture	92	%	Triangular	85,92,98	26
	Cake Solids	25	%	Triangular	10,22,35	26
	PAC Dosage	4	g/kg	Uniform	3-5	19, 26
	Electricity	0.0525	kWh/kg	Uniform	0.035-0.070	35

*Range values for normal distributions include the mean value and standard deviation. For uniform distributions, the minimum and maximum values are given. For triangle distributions, the minimum, most likely, and maximum values are given.

Both the AnMBR and MMC PHA production process begin with fermentation of the influent primary sludge to produce VFA. For this study the HRT and SRT of the fermentation phase was set to 3 days in order to maximize VFA production and minimize loss of VFA to methanogenesis. This was based on the findings of Miron et al. (2000) who determined that for anaerobic digestion of primary sludge under mesophilic conditions, approximately 40% of hydrolysis and 15% of acidogenesis was achieved within a 3 day retention time, with little increase between 3-8 days. Retention times beyond 8 days led to methanogenic activity and thus loss of VFA. This is also consistent with the results presented in Chapter 5 of this dissertation, in which a 3.4 day SRT at 37°C was determined to be optimum for VFA production from the organic fraction of municipal solid waste (OFMSW). Thus, for the baseline condition of both processes, with an HRT/SRT of 3 days, it was assumed that 15% of influent COD was converted to VFA during fermentation. Other studies have suggested that up to 60% VFA production can be achieved during fermentation of municipal sludge (Valentino et al., 2017). This was taken into consideration in the uncertainty analysis of this study. Energy for heating in the fermentation phase was calculated based as the amount of energy needed to heat water from 20°C to 37°C. Energy for mixing was calculated based on values from Metcalf and Eddy (2003).

For the AnMBR option, a submerged membrane bioreactor directly followed the fermentation phase. Values for HRT, SRT, COD removal, methane yield, and biogas quality were determined based on literature values. The membrane was model as polyethylene terephthalate (PET) hollow fiber, with a baseline lifetime of 10 years. To calculate membrane size, a baseline operating flux was set to 12 LMH. Sodium hypochlorite (NaOCl, 12.5% w/w) and citric acid (100% w/w) were included for membrane cleaning, with annual consumptions based on those reported in Shoener et al. (2016). Energy input for membrane filtration was determined based on literature values reported in Martin et al. (2011). Energy for mixing was calculated based on values from Metcalf and Eddy (2003).

For the PHA process, separation of the solid and liquid fractions of the fermented primary sludge was carried out via membrane filtration. The same, material, lifetime, flux, and energy values assumed for membrane configuration in the AnMBR were utilized here. After membrane filtration, the concentrated solids stream was sent to a conventional anaerobic digester for methane recovery. It was assumed that solids would be concentrated up to 10 times and, thus dilution water would needed to adjust the solids content of the anaerobic digestion phase to

approximately 10 g/L. This dilution water was assumed to come from recovered effluent from the biomass selection phase and recovered centrate from waste sludge dewatering.

Following membrane filtration in the PHA production process, the VFA-rich permeate is sent on to a biomass selection phase and PHA accumulation phase. The split fraction for these two streams was calculated based on biomass production in the selection phase and the required food to microorganism (F:M) ratio for the PHA accumulation phase. For the baseline condition, 27% of the feed stream was sent to the biomass selection phase while the remaining 73% was utilized in the PHA accumulation phase. In both the biomass selection and PHA accumulation phase it was assumed all the available VFA was consumed.

Values for HRT, SRT, biomass yield, PHA storage yield, aeration requirement, oxygen transfer efficiency (OTE), and aeration efficiency in the biomass selection and PHA accumulation phases were determined from literature values as shown in Table 6.1. Following PHA accumulation, the PHA-rich biomass was assumed to be dewatered via solid-bowl centrifugation with polyaluminum chloride (PACl) addition. Values for PACl dosage, typical solids capture efficiency, and cake solids percent were based on values for solid-bowl centrifugation from Metcalf and Eddy (2003). Electricity requirements were based on the range reported by Wang et al. (2007).

Finally, for both processes recovered methane was assumed to be utilized to offset electricity consumption via combined heat and power, with a CHP efficiency of 70%. Sludge dewatering for both anaerobic waste sludge and PHA accumulation biomass was assumed to be carried out via solid-bowl centrifugation with PACl addition. Again, PACl dosage, typical solids capture efficiency, and cake solids percent were based on values from Metcalf and Eddy (2003), and electricity requirements were calculated based on the range reported by Wang et al. (2007).

Life cycle assessment

Goal and scope definition

A comparative LCA of the two processes was carried out in accordance with the ISO14040 framework (ISO 14040 and ILCD guidelines: European Commission Joint Research Center, 2010). The functional unit for this analysis was 1 m^3 of influent primary sludge. Figure 6.2 shows the assumed system boundary. For this study, only the operational phase was

considered as was done in Morgan-Sagastume et al. (2016), where it was noted that construction of facilities, vehicles, and other infrastructure generally contribute a minimal fraction of total environmental impacts. Also, consistent with Morgan-Sagastume et al. (2016), a PHA extraction phase was not included within the system boundary. Although it is expected that downstream processing of PHA biomass and PHA extraction will contribute significantly to environmental impacts of MMC PHA production, the choice of extraction method will be dependent on the properties of the recovered PHA and the intended application (Morgan-Sagastume et al., 2016; Valentino et al., 2017). Therefore, as a starting point for the comparison of primary sludge treatment via AnMBR and MMC PHA production, the PHA extraction phase was not included in this study. However, future work should include consideration of the environmental impacts associated with PHA extraction. Both first and second order environmental impacts were considered, in which first order impacts included direct emissions to water, air, and land coming from the two treatment processes themselves, and second order impacts included emissions from off-site processes such as grid electricity production, materials and chemical production, transportation, landfilling, and avoided electricity consumption from methane recovery and utilization, as well as avoided petroleum plastics production with recovered PHA.

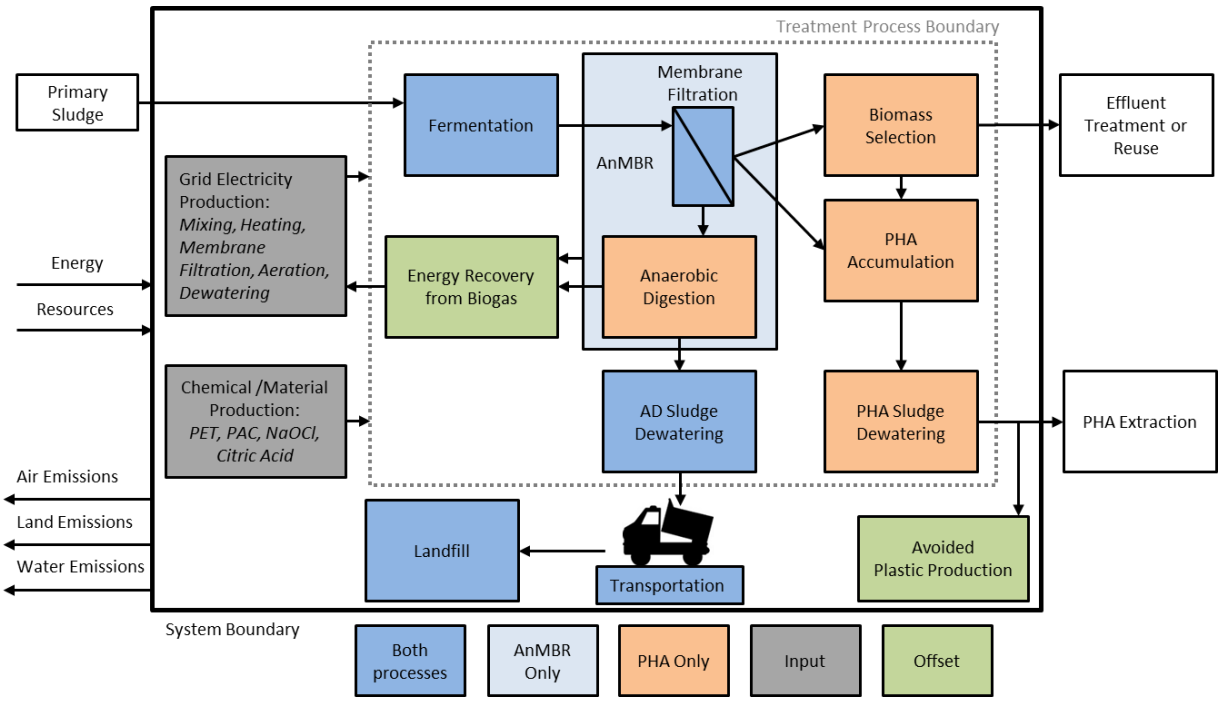


Figure 6.2. System boundary for LCA comparison of primary sludge treatment via AnMBR and MMC PHA production.

Life cycle inventory (LCI)

Life cycle inventory data (LCI) for all materials and processes were collected using the European reference Life Cycle Database (ELCD) v3.2 and Ecoinvent v3, as listed in Table 6.2, with the exception of PACl. LCI data for PACl was obtained from Sharaai et al. (2010). The impact of effluent dissolved methane on GWP was also considered using the 20 year GWP of 84 kg CO_2 eq (IPCC, 2014). For the PHA process, displacement of two different petroleum based polymers was considered: polystyrene (PS) and polypropylene (PP) (Koller et al., 2013).

Table 6.2. Life cycle inventory description for all materials and processes included in LCA.

Parameter	Units	Source
Electricity	kWh	ELCD: Electricity grid mix 1kV-60kV, consumption mix, at consumer, AC, EU-27
PACl	kg	Sharaai et al., 2010
NaOCl	L	Ecoinvent: Sodium hypochlorite, w/o water, 15% solution state {GLO}|market for|Alloc Def, U
Citric acid	L	Ecoinvent: Citric acid {GLO}| market for | Alloc Def, U
Polypropylene	kg	ELCD: Polypropylene granulate (PP), production mix, at plant - RER
Polystyrene	kg	ELCD: Polystyrene expandable granulate (EPS), production mix, at plant - RER
Transportation	t*km	ELCD: Lorry transport, Euro 0,1,2,3,4 mix, 22 t total weight, 17.3 t max payload
Waste landfilling	kg	ELCD: Landfill of biodegradable waste, at landfill site, landfill including landfill gas utilization & leachate treatment & w/o collection, transport & pretreatment

Life cycle impact assessment (LCIA)

The environmental impact of all raw materials and emissions, as quantified in the LCI, was characterized across ten categories: global warming potential (GWP), fossil fuel depletion, ecotoxicity, ozone depletion, respiratory effects, smog, eutrophication, human health cancer, human health non-cancer, and acidification, using the Tool for the Reduction and Assessment of Chemical and other Environmental Impacts (Traci v2.1).

Operating Cost Analysis

An initial cost analysis was conducted taking into account only the cost of operating inputs, inducing energy and material inputs, as well as the potential cost offsets resulting from recovered methane and PHA. Cost values for all operating inputs and offsets were collected from literature as shown in Table 6.3. Resulting net operating cost values are reported as present worth, assuming a rate of return of 10% per year over a lifetime of 20 years.

Table 6.3. Cost values for operating inputs and resulting offsets.

Parameter	Cost	Units	Source
Electricity	0.101	$/kWh	US Energy Information Administration, 2017
PACl	0.25	$/kg	Alibaba.com, 2017
NaOCl	0.22	$/L	Shoener et al., 2016
Citric acid	0.14	$/L	Shoener et al., 2016
PHA	2.3-5.3	$/kg	Valentino et al., 2017
Transportation	$2.26x10^{-4}$	$/kg	Assuming 20 km at 8 mpg and diesel price of $2.5/gal (US EIA, 2016)
Waste landfilling	50	$/ton	US EPA, 2015

Uncertainty and sensitivity analysis

Monte Carlo simulation (3000 trials) was used to evaluate uncertainty for 23 input parameters and to quantify the range of potential environmental impact and net operational cost for the two treatment processes. Input values and probability distributions were determined based on literature data as summarized in Table 6.1. Sensitivity analysis was conducted to evaluate how individual model input parameters impacted model outputs, specifically net operating cost and net GWP. The sensitivity of individual process parameters was quantified by calculating the change in resulting net operating cost and net GWP compared to the initially established baseline condition.

Results and Discussion

Environmental Impact and Net Operating Cost of Baseline Condition

The net operating cost and environmental impacts of the AnMBR and PHA production process for replacing either PP or PS plastics were evaluated for the baseline condition as described in Table 6.1. Results are shown in Figure 6.3 where values are represented as a percentage of the highest value observed in each impact category. Overall, the AnMBR process displayed better performance (indicated by negative values in Figure 6.3) for the majority of environmental impact categories with the exception of fossil fuel depletion, and ecotoxicity in the case of polystyrene displacement with PHA. In the categories of GWP, ozone depletion, respiratory effect and smog, the AnMBR process was net negative, while the PHA production process was net positive. Both processes resulted in a negative net operating cost indicating that the economic value of the recovered methane and PHA was able to offset estimated operating costs in either process. The PHA production resulted in a more negative net operating cost

compared to the AnMBR process owing to the high average selling price of PHA, $3.8/kg (Valentino, et al., 2017). These results suggest that compared to AnMBR treatment, PHA production from primary sludge has the potential to be a more economically favourable option, however, a more comprehensive techno-economic analysis is needed, with consideration of capital costs and PHA extraction, to verify this. However, in terms of environmental impact, primary sludge treatment via AnMBR rather than PHA production appears to be the more environmentally sustainable option.

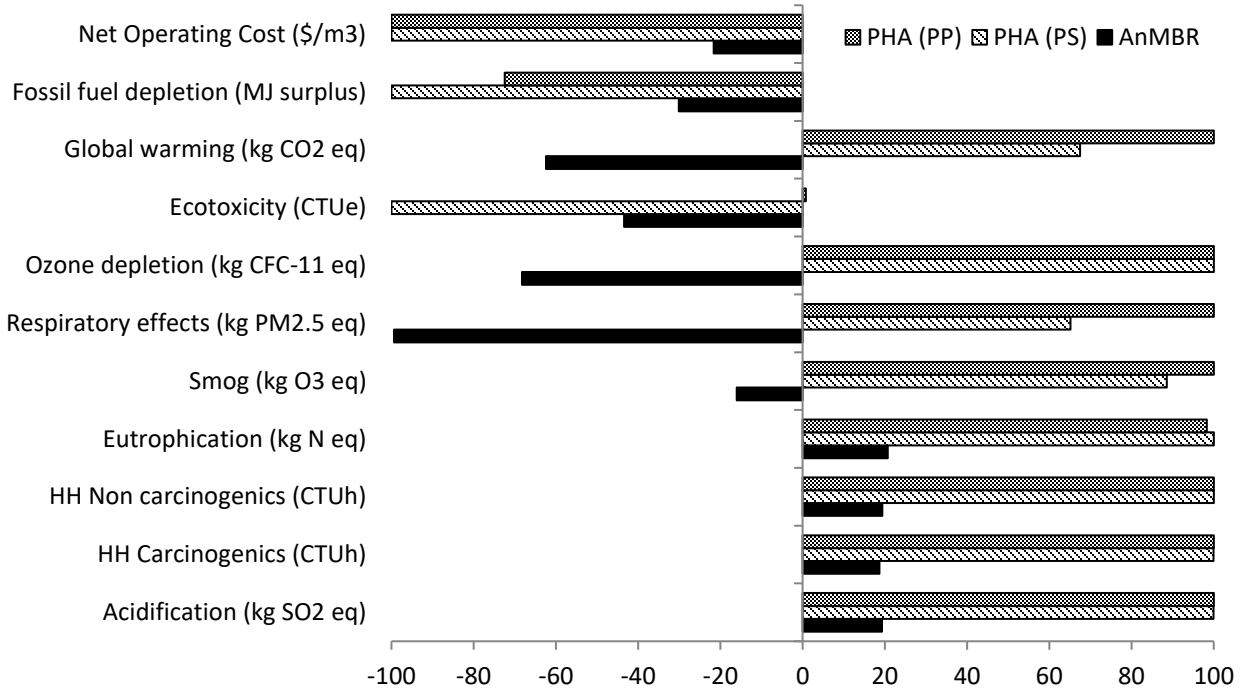


Figure 6.3. Life cycle impact assessment results for the baseline condition.

For both processes, electricity consumption accounted for the majority of operational costs and environmental burden. Figure 6.4 shows the distribution of the various inputs and outputs of each process on net operational cost, GWP, fossil fuel depletion and ecotoxicity. The larger electricity demand for PHA production was the major factor contributing to its higher net GWP relative to the AnMBR process. PHA production also had lower energy recovery as methane and larger burden of sludge landfilling due to greater production of waste anaerobic sludge, which increased the net GWP for PHA production. Nevertheless, the displacement of petroleum based plastic makes the PHA production process more favourable in the category of fossil fuel

depletion. It is important to note that the type of plastic that is displaced by the recovered PHA has important effects on the relative environmental impacts of PHA production. For example, Figures 6.3 and 6.4, show that displacing polystyrene rather than polypropylene results in lower net GWP, fossil fuel depletion, and ecotoxicity, as well as respiratory effects, and smog.

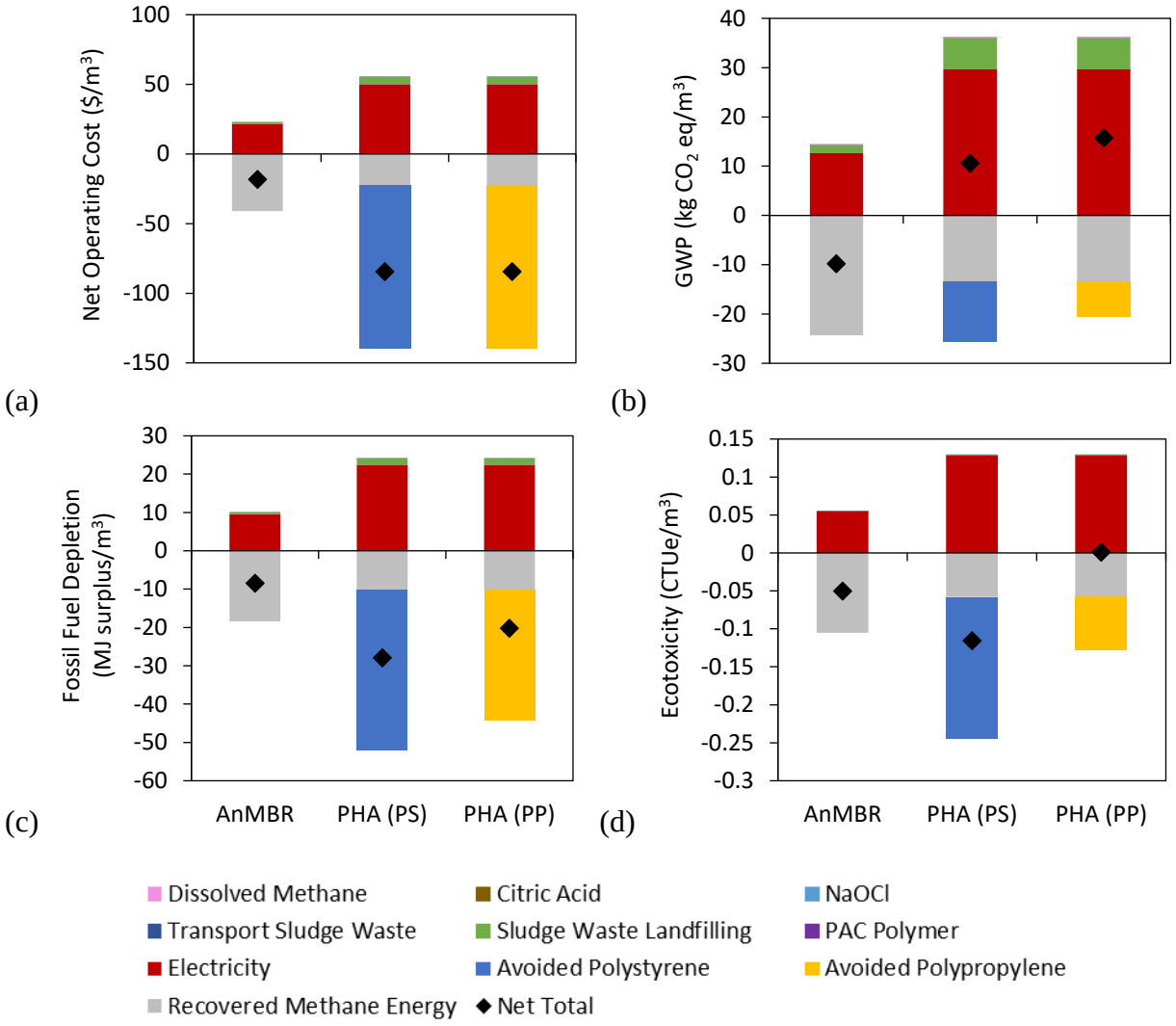


Figure 6.4. Contribution of process inputs and outputs on net (a) Operating Cost, (b) GWP, (c) Fossil Fuel Depletion, and (d) Ecotoxicity for both the AnMBR and MMC PHA production process baseline condition. PS indicates recovered PHA is assumed to displace polystyrene while PP indicates that recovered PHA is assumed to replace polypropylene.

The larger electricity demand associated with the PHA production process is due to the aeration required for the biomass selection and PHA accumulation phases. Aeration was found to account for 57% of the total of electricity demand associated with PHA production, as modelled in this study. Thus, the majority of negative environmental impact associated with the PHA production process is a result of the high energy demand for aeration. Figure 6.5 shows the energy balance for the PHA and AnMBR processes, as well as for conventional mesophilic anaerobic digestion (AD) in which 60% conversion of COD to methane was assumed. In the case of the AnMBR process, the majority of electricity demand was for heating (79%) followed by membrane filtration (12%). However, given the high organic solids concentration of the primary sludge substrate, this analysis indicated that more than enough energy could be recovered as methane to compensate for heating as well as other electricity demands associated with the AnMBR process. Moreover, the analysis indicated that primary sludge treatment via AnMBR could offer a better energy balance compared to conventional anaerobic digestion despite the added energy requirements for membrane filtration.

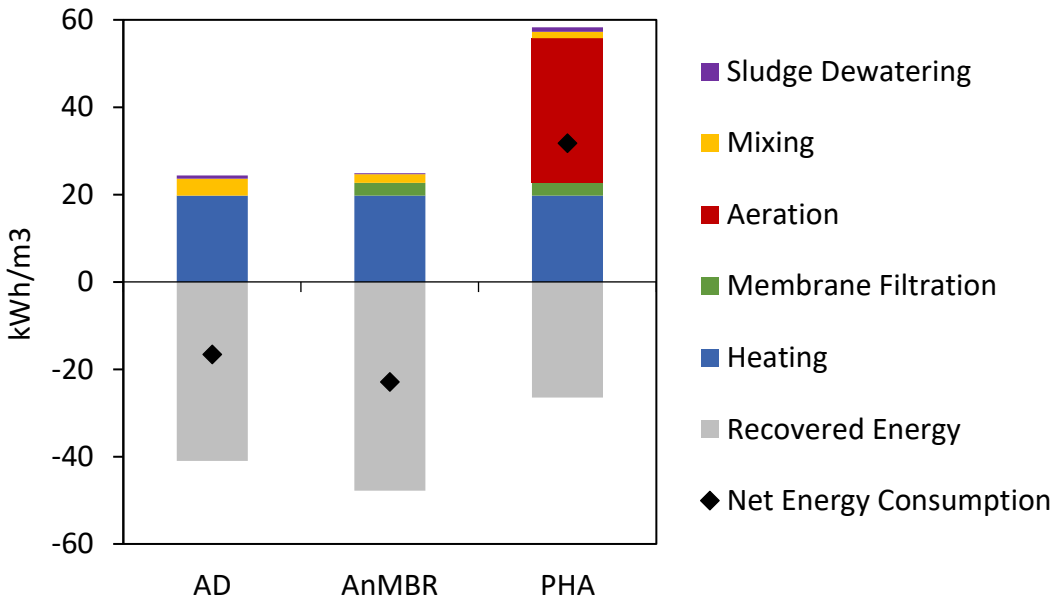


Figure 6.5. Energy balance for the baseline condition of the AnMBR and MMC PHA production process, as well as conventional anaerobic digestion (AD) of primary sludge assuming 60% conversion of COD to methane.

Uncertainty Analysis

Figure 6.6 shows the distribution of net operating cost versus net GWP resulting from random variation (3000 iterations) via Monte Carlo simulation of the 23 process input parameters as described previously in Table 6.1. As can be seen in Figure 6.6, the PHA production processes displayed a much higher degree of variability compared to the AnMBR process. As was observed for the baseline condition, the majority of iterations of the PHA production process resulted in higher net operating cost and higher net GWP compared to the majority of iterations of the AnMBR process. Thus, the AnMBR process appears to be the better option in terms of operating cost and GWP, with the majority of iterations for the AnMBR process (99.1%) resulting in a net negative operating cost and net negative GWP. However, a fraction of the iterations of the PHA process (6.4%) suggest that, it could be possible to achieve a net negative operating cost and net negative GWP for PHA production from primary sludge. Figure 6.7 shows the distribution of values for net operating cost, GWP, fossil fuel depletion, and ecotoxicity resulting from uncertainty analysis of the two processes as well as the initially established baseline value. The majority of iterations for both processes resulted in net negative fossil fuel depletion and ecotoxicity.

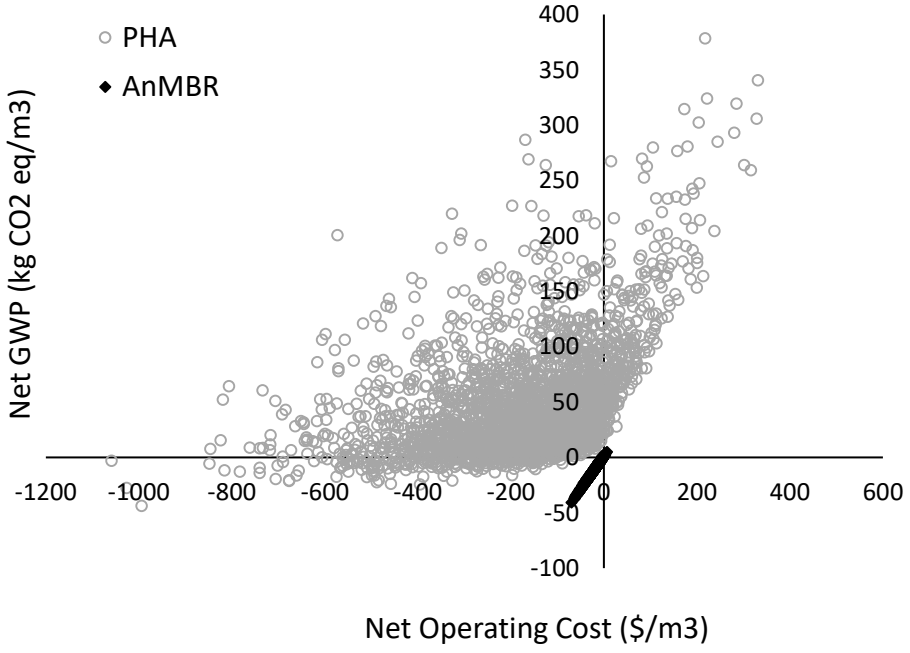


Figure 6.6. Monte Carlo simulation (3000 iterations) displaying the range of potential net operating cost versus net GWP values for the modelled MMC PHA production and AnMBR treatment processes.

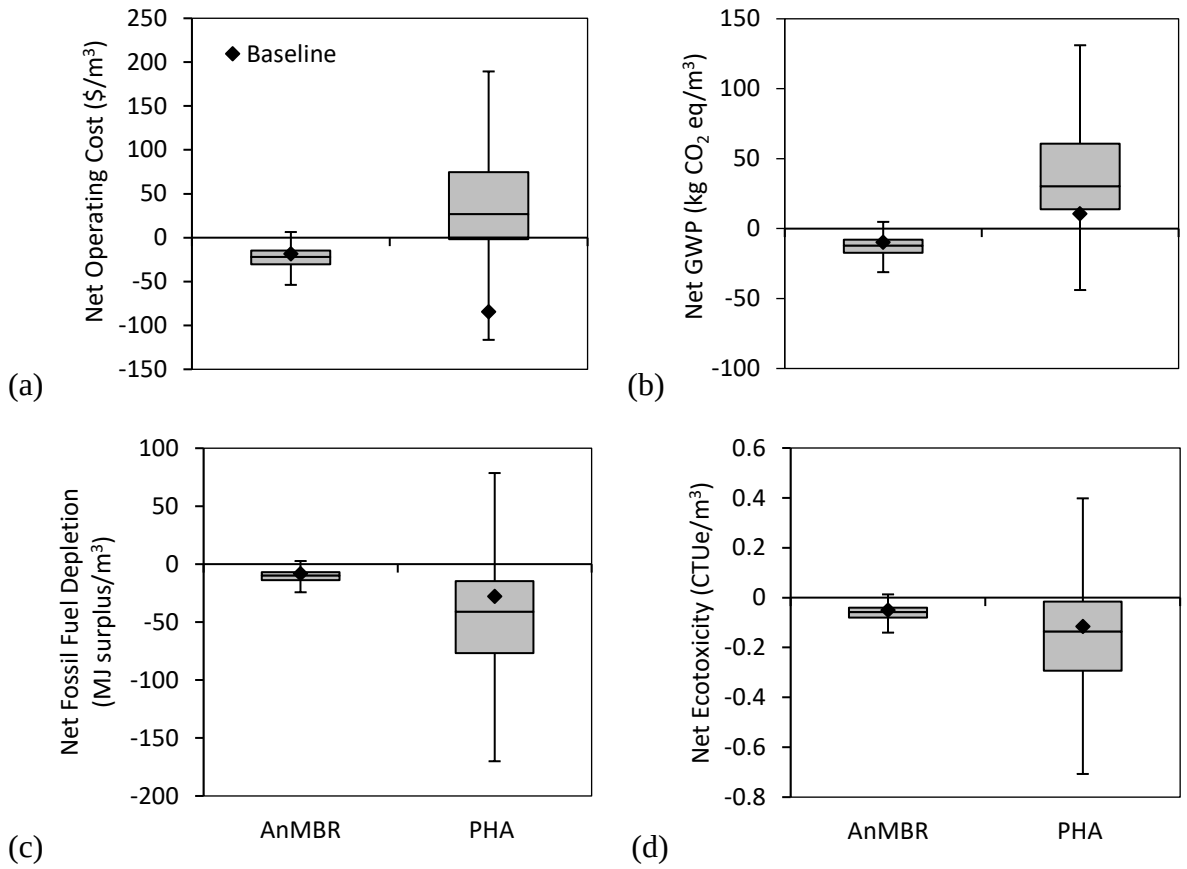


Figure 6.7. Range of values for (a) Net Operating Cost, (b) Net GWP, (c) Net Fossil Fuel Depletion, and (d) Net Ecotoxicity resulting from uncertainty analysis of the modelled AnMBR and MMC PHA production processes. Initial baseline values are also shown. Error bars represent 1.5*IQR.

Overall, analysis of the uncertainty of both process models suggests that primary sludge treatment via AnMBR is the more favorable option in terms of operating cost and GWP, however under optimized conditions, both technologies have the potential to be environmentally sustainable options and achieve a net negative operating cost. Furthermore, PHA production has the potential to offer greater economic benefits due to the high selling price of PHA, however a more detailed economic analysis of the two process including capital costs should be conducted to verify this. Optimization of the PHA production process should focus on reducing its net GWP, and the predicted environmental impacts of both processes should be validated with long-term, large-scale demonstration. Also, the additional environmental and economic impacts of PHA extraction, which were not included in the scope of this study, should be considered in future analysis.

Sensitivity Analysis

Sensitivity analysis of the AnMBR and PHA production processes was conducted, by varying one process parameter at a time, in order to elucidate which process parameters had the greatest influence on environmental impact and operating cost for the two processes. Results are presented in Figure 6.8 where values are shown as the difference compared to the initially established baseline condition resulting from variation of a given process parameter according to its corresponding range and probability distribution as described in Table 6.1. For the PHA production process, specific productivity (g PHA/g $VFA_{removed}$) and percent of acidification in the fermentation phase (% of TCOD) had the greatest effect on net operating cost. As would be expected, maximizing specific productivity and the amount of VFA substrate available for conversion to PHA will resulted in greater PHA production and thus greater cost offset. However, in terms of GWP, aeration efficiency (kg O_2/kWh) had the greatest impact on the PHA production process, followed by percent acidification in the fermentation phase, which was negatively correlated with net GWP. This is due to the associated increase in aeration that would be required to convert larger quantities of VFA to PHA. Thus, while higher rates of VFA production are desirable in order to maximize PHA production, it comes at the cost of increased aeration demand. In general, due to the fact that aeration accounts for the majority of energy demand for PHA production, process parameters associated with aeration, including aeration efficiency as well as aeration required (g O_2/g BOD), and oxygen transfer efficiency (OTE) had a large impact on GWP of the PHA production process. Lower aeration requirement and higher oxygen transfer and aeration efficiencies will result in lower GWP and lower operating cost, and vice versa. Overall, optimization of aeration parameters in order to minimize energy demand was observed to be critical in order to achieve a net negative GWP for the PHA production process. For future research, one interesting possibility to overcome the issue of aeration could be the use of phototrophic PHA accumulating microorganisms, including some species of algae, which do not require aeration (Fradinho et al, 2014).

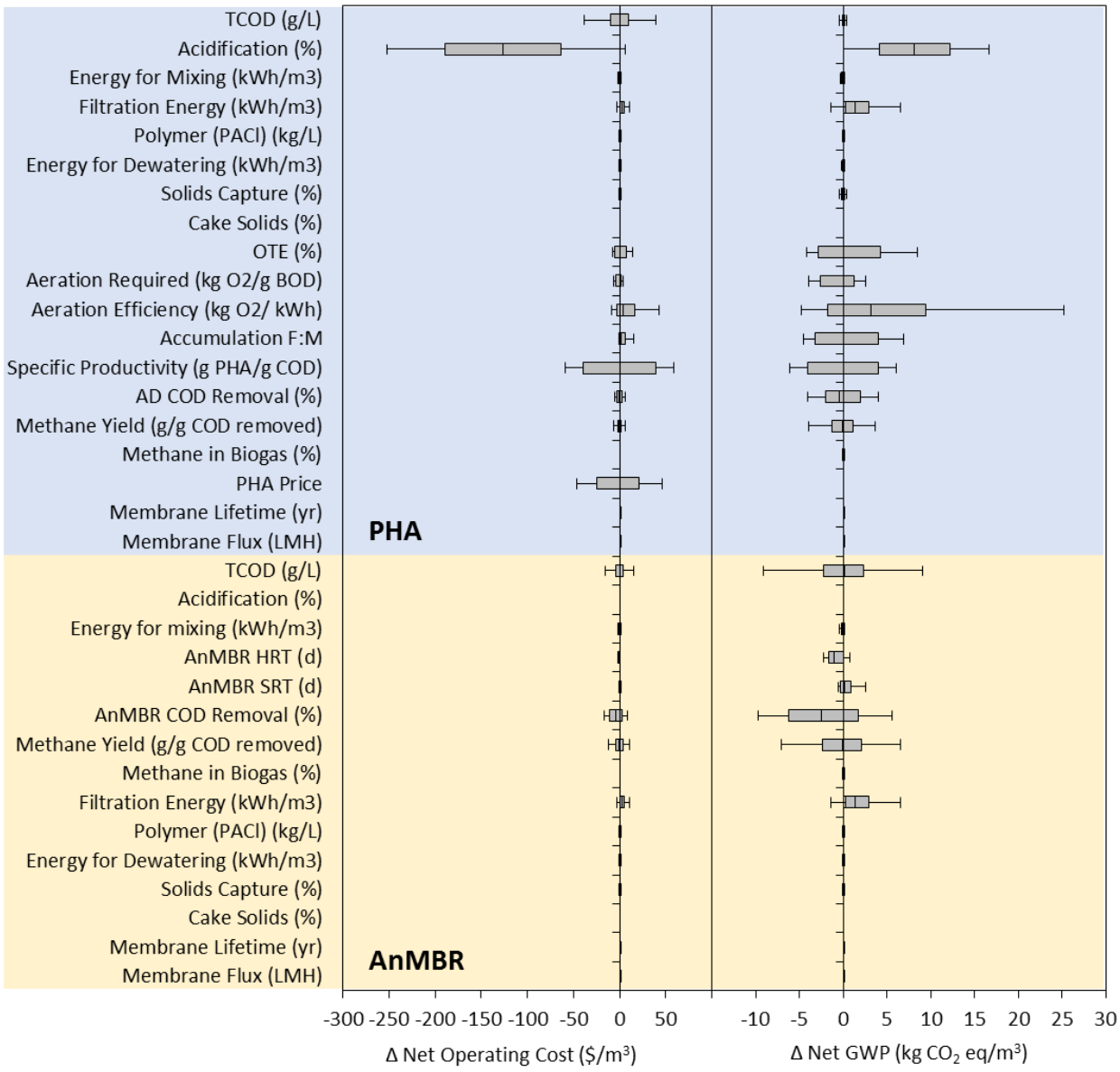


Figure 6.8. Sensitivity of model process parameters on net operational cost and net GWP for the PHA production and AnMBR processes. Values are the change in value relative to the baseline condition.

Other parameters that had a notable impact on net GWP of the PHA production process were the food to microorganism ratio (F:M) in the PHA accumulation phase as well as membrane filtration energy, and COD removal and methane yield in the anaerobic digestion phase. In this study, F:M was used to dictate the fraction of VFA-rich fermentation effluent that was sent to biomass selection and PHA accumulation. As such, a lower F:M in the accumulation phase increased the faction of available VFA sent to the biomass selection phase in order to

produce a sufficient amount of biomass for the accumulation phase. In contrast a higher F:M increased the faction of available VFA sent to the PHA accumulation phase resulting in greater PHA production. A wide range of feed strategies and F:M ratios in the PHA accumulation phase have been reported in the literature. Since PHA yield and aeration requirements were linked to F:M in this process model, variation in F:M had a notable impact on operating cost and GWP. In terms of membrane filtration, higher energy demands for membrane filtration lead to higher cost and GWP. In the case of PHA production, however, the impact of energy demand for membrane filtration energy was not as significant as the impacts associated with aeration. Finally, regarding anaerobic digestion COD removal and methane yield, maximizing methane recovery will help to offset the large energy demand associated with PHA production, therefore optimization of the anaerobic digestion phase of this process is also important.

Similarly, in the AnMBR process, both net operating cost and net GWP were impacted most notably by variation in COD removal and methane yield as well as initial total COD (TCOD). Higher concentrations of initial TCOD means more potential for methane production, and achieving a higher COD removal leads to greater methane recovery. Membrane filtration energy also had a notable impact on net operating cost and net GWP of the AnMBR process. Just as in the case of PHA production, higher energy demands for membrane filtration lead to higher operating cost and GWP. While this study suggests that more than enough energy can be recovered as methane to offset the energy demand for membrane filtration in the AnMBR, this should be validated with large-scale, long-term demonstration.

In general, AnMBRs have been shown to be able to achieve greater COD removal compared to conventional anaerobic digestion due to the retention of solids via the membrane. Therefore, it could be advantageous to combined PHA production with AnMBR treatment in order to recover more energy to offset the high energy demands associated with aeration for PHA production, as well as to reduce the amount of waste sludge produced and the associated economic and environmental burdens of waste sludge handling and disposal. If 90% conversion of waste COD to methane is assumed for the baseline condition of the PHA production process, net energy consumption and net GWP would decrease by 68% (32 to 10 kWh/m^3) and 152% (11 to -6 kg CO_2 eq/m^3), respectively, resulting in a net negative GWP for the PHA production process. Thus, utilization of AnMBR technology as an alternative to conventional anaerobic

digestion has potential for improving the energy balance and environmental sustainability of wastewater treatment processes. Currently, few large scale AnMBR treatment processes are online, however, with future developments in AnMBR research and membrane technology, AnMBRs could become a more widely used technology in wastewater treatment. AnMBRs would be particularly advantageous in areas with limited land availability, such as densely populated urban areas, where there may be additional pressures to expand plant capacity in order to accommodate a growing population, and/or limited land available for waste sludge disposal. Overall, considering the high selling price of PHA, and the positive energy balance associated with AnMBRs, a combination of the two technologies may be a promising approach for managing sewage sludge for resource recovery.

Conclusions

This study applied LCA methodology to evaluate and compare the environmental impacts of two emerging technologies for resource recovery from sewage sludge: methane recovery via AnMBR treatment and bioplastic recovery from MMC PHA production. Models of the two processes were developed based on mass and energy balances using process parameters found in the scientific literature. Evaluation of the baseline condition for each process indicated that AnMBR technology was the more environmentally sustainable option resulting in net negative impact in 6 out of the 10 environmental impact categories considered. In the case of PHA production, the high energy requirements for aeration negatively impacted the global warming potential of the PHA process, although it performed better in the impact categories of fossil fuel depletion and ecotoxicity compared to the AnMBR process. Electricity consumption was found to be the largest contributor to overall environmental impact and operating cost for both processes. However, in the case of treatment via AnMBR, it was estimated that enough energy could be recovered as methane to offset operational energy requirements and achieve a positive energy balance and negative net operating cost. Considering the variability of the process parameters used to model the two processes, uncertainty analysis suggested that it may be possible to achieve net negative GWP for MMC PHA production from primary sludge. Sensitivity analysis revealed that parameter most influential to GWP of PHA production was aeration efficiency. In general, efforts to maximize PHA yield while minimizing energy associated with aeration should be investigated in order to improve both the net operational costs and net GWP of MMC PHA production. Furthermore, due to the high selling price of PHA, the

PHA production process has the potential to substantially offset operating costs associated with its production. Combining MMC PHA production with AnMBR treatment of resulting waste solids could be an advantageous option for improving the energy balance, operating costs, and GWP potential of PHA production. Future analyses should consider the impacts and costs associated with PHA extraction as well as construction, maintenance, and end-of-life, for the two processes. In addition, model and LCA predictions should be validated with long-term, large-scale implementation and demonstration of the two technologies.

References

1. Aliaba.com. (2017). Polyaluminum chloride price.
2. Bugnicourt, E., Cinelli, P., Lazzeri, A., & Alvarez, V. (2014). Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging. *eXPRESS Polymer Letters*, *8*, 791–808.
3. Chang, S. (2014). Anaerobic Membrane Bioreactors (AnMBR) for Wastewater Treatment. *Advances in Chemical Engineering and Science*, *4*(1), 56–61.
4. Dong, Q., Parker, W., & Dagnew, M. (2016). Influence of SRT and HRT on Bioprocess Performance in Anaerobic Membrane Bioreactors Treating Municipal Wastewater. *Water Environment Research*, *88*(2), 158–167.
5. Fallis, A. (2013). *Biosolids Treatment Processes*. *Journal of Chemical Information and Modeling* (6th ed., Vol. 53). Springer.
6. Fernández-Dacosta, C., Posada, J. A., Kleerebezem, R., Cuellar, M. C., & Ramirez, A. (2015). Microbial community-based polyhydroxyalkanoates (PHAs) production from wastewater: Techno-economic analysis and ex-ante environmental assessment. *Bioresource Technology*, *185*, 368–377.
7. Fradinho, J. C., Oehmen, A., & Reis, M. A. M. (2014). Photosynthetic mixed culture polyhydroxyalkanoate (PHA) production from individual and mixed volatile fatty acids (VFAs): Substrate preferences and co-substrate uptake. *Journal of Biotechnology*, *185*, 19–27.
8. Ghyoot, W. R., & Verstraete, W. H. (1997). Coupling membrane filtration to anaerobic primary sludge digestion. *Environmental Technology*, *18*(6), 569–580.
9. Gurieff, N., & Lant, P. (2007). Comparative life cycle assessment and financial analysis of mixed culture polyhydroxyalkanoate production, *98*, 3393–3403.
10. IPCC. (2014). *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. *Climate Change 2014: Synthesis Report*. Geneva, Switzerland.
11. JRC-IES. (2010). ILCD handbook. International reference life cycle data system. General guide for life cycle assessment. 1st ed. European Union
12. Koller, M., Sandholzer, D., Salerno, A., Braunegg, G., & Narodoslawsky, M. (2013). Biopolymer from industrial residues: Life cycle assessment of poly(hydroxyalkanoates) from whey. *Resources, Conservation and Recycling*, *73*, 64–71.
13. Korkakaki, E., Mulders, M., Veeken, A., Rozendal, R., van Loosdrecht, M. C. M., & Kleerebezem, R. (2016). PHA production from the organic fraction of municipal solid waste (OFMSW): Overcoming the inhibitory matrix. *Water Research*, *96*, 74–83.
14. Martin, I., Pidou, M., Soares, A., Judd, S., & Jefferson, B. (2011). Modelling the energy demands of aerobic and anaerobic membrane bioreactors for wastewater treatment *Environmental Technology*, *32*(9), 921–932
15. Martin-Ryals A. (2017) Advancing Wastewater Valorization using Anaerobic Membrane Bioreactors with Bioaugmentation and Ion-Exchange, and Bio-Plastic Production. PhD Dissertation
16. Meabe, E., Déléris, S., Soroa, S., & Sancho, L. (2013). Performance of anaerobic membrane bioreactor for sewage sludge treatment: Mesophilic and thermophilic processes. *Journal of Membrane Science*, *446*, 26–33
17. Miron, Y., Lier, J. B. V. A. N., & Lettinga, G. (2000). the Role of Sludge Retention Time in the Hydrolysis and Acidification of Lipids , Carbohydrates and Proteins During Digestion of Primary Sludge in Cstr Systems. *Water Research*, *34*(5).
18. Mo, W., & Zhang, Q. (2013). Energy-nutrients-water nexus: Integrated resource recovery in municipal wastewater treatment plants. *Journal of Environmental Management*, *127*(30), 255–267.

19. Morgan-Sagastume, F., Heimersson, S., Laera, G., Werker, A., & Svanstrom, M. (2016). Techno-environmental assessment of integrating polyhydroxyalkanoate (PHA) production with services of municipal wastewater treatment. *Journal of Cleaner Production*, *137*, 1368–1381.

20. Pierkiel, A., & Lanting, J. (2005). Membrane-coupled anaerobic digestion of municipal sewage sludge. *Water Science & Technology*, *52*(1–2), 253–258.

21. Rittman, B. E., & McCarty, P. L. (2001). *Environmental Biotechnology: Principles and Applications*.

22. Serafim, L. S., Lemos, P. C., Albuquerque, M. G. E., & Reis, M. A. M. (2008). Strategies for PHA production by mixed cultures and renewable waste materials. *Applied Microbiology and Biotechnology*, *81*(4), 615–628.

23. Sharaai, A. H., Mahmood, N. Z., & Sulaiman, A. H. (2012). Life cycle impact assessment (LCIA) using the ecological scarcity (ecopoints) method : A potential impact analysis to potable water production. *World Applied Sciences Journal*, *11*(6), 1391–1402.

24. Shoener, B. D., Zhong, C., Greiner, A. D., O. Khunjar, W., Hong, P.-Y., & Guest, J. S. (2016). Design of anaerobic membrane bioreactors for the valorization of dilute organic carbon waste streams. *Energy & Environmental Science*, *9*(3), 1102–1112.

25. Tamis, J., Lužkov, K., Jiang, Y., Loosdrecht, M. C. M. van, & Kleerebezem, R. (2014). Enrichment of Plasticicumulans acidivorans at pilot-scale for PHA production on industrial wastewater. *Journal of Biotechnology*, *192*(Part A), 161–169.

26. Metcalf and Eddy Inc. (2003). *Wastewater Engineering: Treatment and Reuse* (4th ed.).

27. U.S. Department of Energy. (2016). Combined Heat and Power Basics.

28. U.S. Energy Information Administration. (2016). Gasoline and Diesel Fuel Update.

29. U.S. Energy Information Administration. (2017). Electric Power Monthly: Data for November 2016.

30. U.S. EPA. (2015). Historic Tipping Fees and Commodity Values.

31. U.S. EPA. (2006). Wastewater Management Fact Sheet: Energy Conservation.

32. Valentino, F., Morgan-Sagastume, F., Campanari, S., Villano, M., Werker, A., & Majone, M. (2017). Carbon recovery from wastewater through bioconversion into biodegradable polymers. *New Biotechnology*.

33. Verrecht, B., Maere, T., Nopens, I., Brepols, C., & Judd, S. (2010). The cost of a large-scale hollow fibre MBR. *Water Research*, *44*(18), 5274–5283.

34. Villano, M., Valentino, F., Barbetta, A., Martino, L., Scandola, M., & Majone, M. (2014). Polyhydroxyalkanoates production with mixed microbial cultures: From culture selection to polymer recovery in a high-rate continuous process. *New Biotechnology*, *31*(4), 289–296.

35. Wang, L. K., Shammas, N. K., & Hung, Y. (2007). Biosolids Treatment Processes (6th ed.). Springer.

CHAPTER 7: CONCLUSIONS AND FUTURE RECOMMENDATIONS

In this work, two emerging technologies for resource recovery from organic waste were investigated. The first was a two-phase anaerobic membrane bioreactor (AnMBR) process incorporating bioaugmentation and ion-exchange resin to improve process stability and methane recovery from primary sewage sludge. The second was mixed microbial culture (MMC) polyhydroxyalkanoate (PHA) production from fermented municipal solid waste (OFMSW) with application of clinoptilolite to reduce ammonium-nitrogen concentrations and improve PHA accumulation. The main conclusions from this work are the following:

- Bioaugmentation with hydrolytic and acetogenic bacteria in the acid-phase of the two-phase AnMBR process was an effective means for improving average substrate hydrolysis (25-38%) and average acid-phase acetic acid generation (31-52%) from primary sludge, leading to increased average solids reduction (25-55%) and methane production (10-13%).
- Addition of the strong-base anion-exchange resin Purolite TANEX in the two-phase AnMBR system improved effluent quality reducing effluent COD concentrations by 48-75% under normal operating conditions.
- Addition of Purolite TANEX also improved reactor recovery after organic shock-loading by providing relief to the microbial community via sorption of excess acetic acid, resulting in up to 58% faster recovery of methane production compared to operation without TANEX.
- The feasibility of MMC PHA production from hydrolyzed OFMSW was demonstrated, archiving a maximum PHA yield of 38% of VSS under low ammonium-nitrogen conditions.
- Optimum fermentation parameters for maximum VFA production from OFMSW were determined to be 5.4% solids, 37ºC, and 3.4 day HRT.
- Addition of the natural zeolite, clinoptilolite, was determined an effective means for reducing ammonium-nitrogen concentrations in hydrolyzed OFMSW, resulting in 29% greater PHA yield compared to untreated hydrolyzed OFMSW.

- LCA of methane recovery via AnMBR treatment versus MMC PHA production suggested that under optimized conditions, both processes have the potential to be environmentally sustainable options for resource recovery from sewage sludge.
- LCA results suggested that sewage sludge treatment via AnMBR could provide a positive energy balance resulting an overall net negative operating cost and GWP, while optimization of the PHA process should focus on maximizing PHA yield and minimizing energy demand for aeration in order to improve GWP.

Future work regarding bioaugmentation should include optimization of the bioaugmentation culture composition and dosing strategy, in order to improve the effectiveness of bioaugmentation and minimize associated operating costs. In addition, the impact of increased hydrolysis and solids removal via bioaugmentation on cake formation and membrane fouling in the AnMBR should be evaluated, as membrane fouling is a significant challenge for AnMBR processes. It may also be interesting to investigated bioaugmentation with hydrolytic and acetogenic microorganisms in the fermentation phase of the PHA production process. For both AnMBR treatment, and PHA production effective conversion of particulate organics to volatile fatty acids is a critical step that will impact the amount of methane and/or PHA that can be recovered. Thus, bioaugmentation with hydrolytic microorganisms may also be an effective strategy for increasing VFA generation for PHA production.

Regarding application of ion-exchange resin in the AnMBR system, the TANEX resin was not able to mitigate pH fluctuation associated with the investigated organic-shock load. Therefore, future work should investigate the effect of adding a cationic resin that could be used to help buffer against sudden deceases in pH caused by hydrogen ions dissociating from VFAs. Also, the impacts and potential benefits of resin addition on permeate flux and membrane fouling should be investigated. In addition, it could be interesting to investigate the application of TANEX or other anion-exchange resins in the PHA production process as a means for harvesting VFAs from complex organic feedstocks. This could potentially reduce complications regarding particulate COD, nutrients, and other toxicants that can limit PHA production from complex organic wastes.

Future work regarding the PHA study should also include optimization of process parameters in the biomass selection and PHA accumulation phases, including F:M ratio, feeding

frequency, and SRT, in order to maximize PHA yield. In addition, application of clinoptilolite in the PHA production process should be further developed including optimization of dosage and evaluation of regeneration strategies for nitrogen recovery.

Finally, future work regarding the LCA comparison of sewage sludge treatment via AnMBR and PHA production for resource recovery should include consideration of the economic and environmental impacts associated with PHA extraction as well as construction, maintenance, and end-of-life, for the two processes. In addition, the results and predictions presented in the LCA study should be validated with large-scale implementation and demonstration of the two technologies.

APPENDIX A: FIGURE DATA

Table A.1. Influent and Acid-phase soluble COD (mg/L) data for Chapter 3 Figure 3.4a.

Bioaugmentation 1		**No Bioaugmentation**			**Bioaugmentation 2**					
Influent	*Acid-phase*	*Influent*	*Acid-phase*		*Influent*		*Acid-phase*			
6605.277	12824.8	6843.305	12168.405	6756.68	9432.466	5178.219	14347.32	8939.47	7393.525	6632.334
6412.4	12824.8	6605.277	9882.4293	6738.668	9316.513	5097.602	14109.237	8845.478	7385.422	6601.542
5880.64	12011.52	6384.49	9342.599	6663.8707	8054.264	4868.023	14109.237	8726.265	7368.898	6561.06
4254.7505	11823.84	6241.34	8807.036	6626.472	7895.29	4578.023	12464.043	8663.01	7348.88	6533.128
4195.2435	11761.28	6025.383	8683.3987	6575.5235	7886.426	4520.5864	12331.291	8649.42	7304.608	6508.863
4035.12	11603.865	6025.383	8628.515	6499.01	7853.343	4447.24	12205.554	8646.618	7301.828	6376.18
3986.969	11385.92	5882.148	8628.515	6402.08	7811.241	4380.2796	11712.512	8582.384	7260.428	6341.436
	11260.8	5742.134	8569.008	6397.0025	7467.246	4354.7544	11467.014	8564.698	7220.409	6138.873
	11187.316	5710.266	8431.016	6384.49	7460.904	4354.7544	10844.46	8517.58	7155.798	6108.87
	11073.12	5611.162	8330.98	6337.4955	7380.065	4150.76	10844.46	8439	7155.798	6072.583
	11073.12	5567.031	8211.966	6252.4853	7294.263	3789.596	10680.572	8343.064	7154.363	6001.951
	10830.274	5280.561	7973.938	6248.235	7278.62	3647.682	10389.72	8336.616	7141.16	5951.571
	10760.32	5108.679	7923.0213	6241.34	7257.336	3647.682	10050.882	8325.448	7132.52	5922.95
	10711.26	5087.274	7914.431	6155.45	6999.596	3619.035	9825.536	8036.886	7121.452	5701.835
	10592.246	4965.444	7741.5947	6040.93	6967.13		9802.83	8023.118	7116.6	4983.909
	10384.96	4946.01	7616.896	6028.0933	6529.988		9655.428	7976.18	7047.457	4983.909
	10354.218	4850.856	7616.896	6012.3	6517.32		9557.316	7895.29	7025.14	4875.053
	10235.204	4784.2	7449.2427	5915.8973	6481.56		9519.35	7837.858	7016.113	4838.768
	9997.176	4719.476	7378.868	5854.7573	6430.545		9464.308	7761.344	7001.572	4584.771
	9997.176	4654.752	7319.361	5840.52	6257.7		9371.3	7747.72	6993.9	4354.754
	9937.669	4654.752	7259.854	5579.3093	6170.15		9345.54	7745.362	6993.9	4341.366
	9818.655	4590.028	7214.76	5467.1133	6169.52		9335.793	7668.75	6984.662	4220.866
	9696.8	4557.666	7187.452	5467.1133	6104.325		9324.796	7658.203	6971.046	
	9461.613	4525.304	7111.0865	5093.1267	5924.118		9241.764	7655.744	6909.12	
	9342.599	4460.58	7075.256	4983.9093	5855.02		9092.276	7561.768	6851.11	
	9133.76	3843.04	7042.98	4911.3387	5504.488		9091.879	7515.375	6798.176	
	8883.52	3808.448	6928.46		5343.763		9085.941	7511.14	6790.288	
	8330.98	3800	6776.0667		5296.304		9045.772	7486.556	6786.294	
	8132.8	3777.554	6756.68		5284.84		8997.48	7406.21	6677.08	

Table A.2. Influent and Acid-phase VFA data (mg COD/L) for Chapter 3 Figure 3.4b-d.

Bioaugmentation 1						No Bioaugmentation						Bioaugmentation 2					
Influent			*Acid-phase*			*Influent*			*Acid-phase*			*Influent*			*Acid-phase*		
Hac	HPr	HBu	Hac	HPr	HBu	Hac	HPr	HBu	Hac	HPr	HBu	Hac	HPr	HBu	Hac	HPr	HBu
692.8	840.9	550.9	1223.7	3651.4	1316.0	748.8	830.5	611.9	838.8	1538.3	967.3	717.4	1329.5	482.7	991.3	2957.8	3046.0
681.1	779.3	525.6	1155.1	3361.2	1283.3	785.7	837.7	644.3	775.3	1359.5	954.7	397.3	636.1	253.3	1038.6	1332.5	2594.8
851.6	954.5	1468.3	1139.9	3352.4	1086.3	835.5	911.7	1126.7	748.8	1357.0	909.5	435.2	728.2	292.5	1789.9	1636.1	2485.9
865.5	914.2	1040.9	1126.3	3181.3	974.4	832.3	919.0	1004.5	748.4	1304.5	888.1	601.2	1056.3	445.4	953.2	3348.6	2482.1
782.8	852.1	640.6	1123.6	3042.4	914.5	850.0	914.6	946.0	735.5	1299.2	835.9	818.6	1475.5	628.0	1630.0	3547.1	2375.7
787.3	859.1	649.8	1119.2	2956.3	854.9	866.1	916.4	913.5	720.8	1229.7	806.2	747.7	1308.4	591.1	1494.5	1423.4	2183.0
			1074.6	2838.2	828.2	204.5	291.5	76.1	714.4	1193.7	803.5	1530.7	3023.8	1353.8	1365.9	3352.0	2081.9
			1047.1	2731.2	816.5	161.6	207.6	61.0	711.1	1143.5	791.2	1263.8	2391.3	1103.8	1047.8	1818.0	1844.1
			1024.3	2463.4	816.0	245.0	374.2	129.4	707.0	1136.5	782.3	660.5	1107.0	525.0	928.3	2633.2	1735.8
			1015.5	2304.0	815.3	267.8	419.3	137.0	687.5	1135.4	770.3	441.1	758.9	1427.4	658.0	1887.2	1337.0
			1011.3	2295.6	795.4	235.1	347.2	120.0	679.7	1123.9	764.0	949.1	1769.7	846.1		1661.5	1180.8
			1006.0	2205.5	792.4	472.1	857.3	291.9	676.5	1123.4	756.0	888.7	1624.0	811.6		1178.3	760.3
			992.9	2197.6	791.9				669.3	1118.1	752.7	785.8	1412.1	719.1			
			975.4	2164.6	787.1				665.5	1116.4	749.9	740.8	1330.2	663.4			
			963.4	2098.6	777.7				661.6	1113.2	746.7						
			949.0	1914.3	766.6				659.5	1108.7	737.9						
			926.7	1800.5	747.0				654.4	1107.8	679.7						
			923.9	1717.6	732.2				651.3	1105.2	665.8						
			908.6	1638.9	724.3				635.4	1103.5	660.5						
			712.0	1452.5	673.9				635.2	1101.9	655.8						
									591.1	1097.2	629.7						
									580.9	1097.0	622.6						
									538.4	1096.0	620.3						
									537.8	1094.7	620.2						
									516.3	1086.2	616.5						
									514.0	1086.1	615.3						
									504.8	1084.1	609.6						
									501.9	1079.0	596.6						
									493.8	1077.7	586.6						
									468.0	1077.3	584.5						
									467.6	1070.7	583.6						
									461.0	1069.1	580.9						
									448.1	1068.2	554.2						
									447.9	1066.7	501.1						
									440.8	1066.4	497.1						
									432.8	1064.1	442.5						
									431.8	1055.2	424.5						
									428.5	1052.0	421.1						
									238.0	1049.0	400.3						
									227.2	1036.4	350.3						

Table A.3. Influent, acid-phase, and methane-phase sulfide (mg/L) data for Chapter 3 Figure 3.5.

Influent		Acid-Phase		Methane-Phase			
Day	S^{2-} (mg/L)	Day	S^{2-} (mg/L)	Day	S^{2-} (mg/L)	Day	S^{2-} (mg/L)
116	18.60	177	31.80	104	63.60	449	43.20
174	35.40	191	22.20	116	87.00	459	39.00
204	23.40	198	28.80	146	96.00	502	30.60
217	30.60	206	25.80	167	94.80	511	30.00
225	21.60	213	32.40	181	87.60	564	18.00
244	31.20	215	23.10	189	93.60	564	29.5, 29.5, 28.1
268	30.60	217	22.20	195	103.80	565	21.60
273	23.40	218	22.20	202	121.20	566	18.00
281	41.40	223	22.20	216	123.00	566	27.9, 30.2, 30.4
296	17.40	226	28.20	218	133.20	567	18.60
302	23.40	235	30.60	220	114.60	568	19.20
330	7.2, 7.8, 14.3	237	41.40	222	123.00	568	24.4, 25.5, 27.6
350	22.0, 23.4, 29.0	255	28.80	224	111.00	569	16.80
359	24.6, 23.5, 24.4	266	38.40	226	115.20	592	20.2, 21.8, 23.5
375	20.6, 19.0, 17.0	279	29.40	228	114.00	594	20.6, 20.1, 20.8
389	47.0, 14.5, 15.1	281	33.00	230	118.20	600	19.7, 20.9, 22.1
410	18.9, 5.7, 17.5	283	33.60	232	141.00	623	20.7, 21.6, 23.8
420	12.3, 13.7, 8.9	287	42.60	234	140.40	627	21.6, 21.9, 23.2
561	15.3, 18.5, 6.5	290	27.60	237	110.40	645	20.6, 22.5, 23.8
566	9.00	292	25.20	240	91.80	647	24.1, 19.4, 22.0
566	12.2, 16.0, 5.3	348	16.9, 18.9, 18.5	245	106.20	648	19.9, 10.3, 7.4
569	9.30	358	16.6, 19.9, 18.4	250	121.20	651	25.4, 25.5, 27.5
569	13.7, 4.9, 13.4	379	28.6, 26.1, 26.3	263	101.40	655	14.3, 12.5, 18.3
585	14.8, 9.1, 15.0	389	9.0, 9.0, 8.5	265	78.00		
600	19.0, 18.4, 7.2	410	16.5, 16.5, 21.0	266	90.00		
634	16.3, 17.8, 13.7	420	19.2, 22.7, 17.8	270	101.40		
		430	11.81, 17.6, 18.7	272	86.50		
		449	14.10	277	94.80		
		459	15.30	279	81.00		
		502	9.60	284	86.40		
		511	9.30	286	88.50		
		564	8.70	288	88.50		
		564	16.4, 17.5, 16.6	289	93.60		
		565	8.10	290	76.50		
		566	10.80	292	75.00		
		566	14.8, 14.0, 15.2	294	92.40		
		567	12.00	295	79.80		
		568	8.40	296	94.80		
		568	13.4, 14.5, 15.8	298	75.00		
		569	8.70	300	70.00		
		592	15.7, 15.7, 12.7	301	99.60		
		594	17.2, 14.6, 16.1	302	99.60		
		600	14.2, 14.8, 14.3	348	30.9, 31.4, 37.2		
		623	16.2, 16.4, 17.8	358	29.6, 29.7, 29.8		
		627	13.9, 15.3, 14.6	379	36.2, 39.0, 39.7		
		645	10.2, 11.4, 11.5	389	32.3, 34.3, 34.6		
		648	12.8, 11.3, 12.0	410	30.1, 29.8, 31.8		
		651	15.2, 14.5, 15.0	420	40.5, 35.7, 41.8		
		655	8.4, 9.8, 12.6	430	37.7, 40.4, 21.1		

Table A.4. Methane, OLR, and COD data for Chapter 3 Figure 3.6.

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
1		1.95	469.40	4378.18	290.14		
2		1.95	469.40				
3		1.95	469.40				
4		1.95	469.40				
5		1.95	469.40				
6		1.95	469.40				
7		1.95	469.40				110.84
8		0.33	469.40				
9		0.33	469.40				
10		0.33	469.40				
11		0.33	469.40				
12		0.33	469.40				
13		0.33	469.40				
14		0.33	469.40				
15		0.33	469.40				
16		0.04	469.40				99.43
17	121.63	0.33	469.40				
18	138.77	0.00	469.40				
19	155.91	0.00	469.40				
20	161.49	0.33	469.40				
21	154.38	0.33	469.40			3977.20	92.91
22	145.63	0.33	469.40				
23	140.30	0.33	469.40				
24	135.83	0.33	469.40			4322.76	84.76
25	137.60	0.33	469.40				
26	119.82	0.14	469.40				
27	120.70	0.33	469.40			4603.12	102.69
28	123.08	0.33	469.40				
29	114.20	0.14	469.40				
30	118.96	0.33	469.40			4224.96	94.54
31	115.57	0.33	469.40				
32	115.84	0.33	469.40				
33	158.24	0.33	469.40	8110.61	605.22	4592.94	89.09
34	196.29	0.29	469.40				
35	233.62	0.26	469.40	6006.15	640.55	4807.99	92.17
36	287.74	0.36	469.40			5529.96	76.81
37	349.72	0.31	469.40				
38	382.44	0.00	469.40			5867.90	125.96
39	464.29	0.18	469.40				
40	536.89	0.33	469.40	7035.34	645.16	8663.60	119.82
41	498.42	0.29	469.40				
42	520.06	0.46	469.40			10168.98	125.96
43	531.67	0.11	469.40				
44	565.95	0.24	469.40			10691.26	119.82
45	555.06	0.11	469.40	8540.72	669.74	9062.99	92.17
46	556.76	0.34	469.40				
47	569.03	0.33	469.40			10752.70	98.31
48	557.95	0.34	469.40				
49	499.02	0.35	469.40	8540.72	860.22	11797.25	107.53
50	503.84	0.42	469.40				
51	490.33	0.31	469.40	8049.16	746.54	10076.82	113.67
52	460.97	0.29	469.40				
53	468.07	0.31	469.40			9523.82	107.53
54	459.57	0.37	469.40				
55	420.53	0.08	469.40			10261.15	98.31
56	393.51	0.41	469.40				

Table A.4. Continued

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
57	430.31	0.34	469.40			13456.24	113.67
58	457.84	0.07	469.40				
59	441.33	0.03	469.40			11920.14	122.89
60	447.21	0.37	469.40				
61	354.68	0.34	469.40	8663.60	1082.95	12288.80	104.45
62	355.44	0.96	469.40				
63	275.85	0.31	469.40			14132.12	125.96
64	272.69	0.96	469.40	8479.27	854.07	13149.02	264.21
65	272.28	0.24	469.40				
66	270.95	0.33	469.40	9216.60	761.91	13886.34	215.05
67	292.16	0.26	469.40				
68	299.55	0.26	469.40			11981.58	79.88
69	296.86	0.29	469.40				
70	293.78	0.24	469.40			12350.24	89.09
71	321.10	0.24	469.40				
72	312.56	0.03	469.40			12964.68	104.45
73	414.07	0.27	469.40				
74	398.80	0.05	469.40			12288.80	64.52
75	576.61	0.27	469.40				
76	610.32	0.03	469.40			12534.58	61.44
77	572.66	0.04	469.40				
78	517.02	0.33	469.40			11674.36	61.44
79	517.31	0.33	469.40				
80	520.28	0.05	469.40			11059.92	58.37
81	458.15	0.05	469.40				
82	423.00	0.30	469.40			11691.60	
83	376.23	0.25	469.40				
84	395.06	0.26	469.40			11992.93	
85	370.13	0.25	469.40			12595.59	103.22
86	393.93	0.30	469.40	5317.60	437.92	11450.54	
87	353.62	0.22	469.40	43090.19	4500.00	12776.39	
88	341.31	0.30	469.40			12113.47	
89	234.06	0.20	469.40			12234.00	111.49
90	246.17	2.18	469.40			11510.81	
91	265.19	1.40	469.40			13258.52	
92	223.79	0.00	469.40				
93	223.46	2.16	469.40			12776.39	301.33
94	258.23	1.63	469.40				
95	244.94	0.70	469.40			15006.23	
96	273.51	2.38	469.40			16633.42	418.85
97	277.25	0.83	469.40			17829.60	325.31
98	273.58	2.04	469.40				
99	296.07	2.77	469.40			20644.80	603.70
100	333.15	1.57	469.40				
101	393.70	0.00	469.40				200.19
102	400.31	0.48	469.40			18392.64	
103	398.11	1.99	469.40	39100.00	4500.00		
104	430.78	1.81	469.40				
105	450.17	2.46	469.40			29152.96	281.52
106	429.31	2.29	469.40			26431.60	
107	475.07	2.90	469.40				
108	466.06	1.21	469.40			31749.20	294.03
109	494.02	1.93	469.40				
110	479.48	0.85	469.40	42384.40	4629.44	28652.48	300.29
111	511.40	3.91	469.40				
112	461.93	0.41	469.40			32280.96	319.06

Table A.4. Continued

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
113	471.23	3.27	469.40			32844.00	
114	471.97	0.70	469.40			36535.04	306.54
115	469.71	2.79	469.40			34095.20	
116	506.66	2.38	469.40			33000.40	334.70
117	545.07	0.35	469.40	46607.20	4566.88	33000.40	269.01
118	580.34	0.35	469.40			26431.60	278.39
119	536.38	0.93	469.40			38630.80	
120	552.17	3.17	469.40			33938.80	
121	548.31	1.39	469.40			37066.80	
122	580.72	1.51	469.40			36754.00	
123	560.99	3.19	469.40			29090.40	
124	600.68	0.93	469.40			31280.00	
125	556.66	1.16	469.40			30028.80	
126	559.92	2.40	469.40			36910.40	
127	566.81	2.43	469.40	48484.00		36284.80	
128	523.44	2.26	469.40	43166.40		31749.20	193.94
129	516.25	2.26	469.40				
130	489.10	0.00	469.40			39412.80	
131	510.67	2.90	469.40				
132	510.18	1.85	469.40			41446.00	
133	502.23	0.41	469.40				
134	499.25	1.62	469.40			38161.60	
135	492.59	2.55	469.40				
136	485.96	1.62	469.40			31905.60	
137	509.51	2.09	469.40				
138	541.39	0.23	469.40			31905.60	
139	531.21	0.00	469.40			33782.40	
140	519.51	1.39	469.40	42384.40		37223.20	
141	434.09	1.62	469.40				
142	408.06	3.01	469.40			32844.00	
143	388.34	2.63	469.40				
144	368.68	1.24	469.40			33469.60	
145	361.87	1.51	469.40				
146	335.05	0.35	469.40	43792.00		37536.00	
147	287.86	1.74	469.40				
148	245.99	1.20	469.40			42540.80	
149	235.35	2.55	469.40				
150	245.78	2.20	469.40			34720.80	
151	234.75	0.23	469.40	36910.40	4035.12		
152	260.56	2.90	469.40			35972.00	344.08
153	291.23	0.00	469.40			34095.20	
154	324.89	2.92	469.40			40976.80	
155	318.70	2.73	469.40			40038.40	
156	335.51	2.68	469.40	50986.40		39100.00	
157	350.26	1.32	469.40			42540.80	
158	380.83	0.82	469.40				
159	417.22	0.93	469.40			25336.80	
160	437.96	0.70	469.40			33156.80	
161	453.37	2.57	469.40			37536.00	294.03
162	437.78	1.79	469.40				
163	485.56	2.06	469.40				
164	481.54	1.87	469.40			30967.20	
165	490.34	1.28	469.40				
166	500.71	3.15	469.40	47232.80	5880.64		
167	552.67	2.45	469.40			30967.20	
168	522.52	0.43	469.40				

Table A.4. Continued

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
169	486.88	2.80	469.40			24711.20	256.50
170	465.62	3.56	469.40	49109.60			
171	468.17	4.28	469.40				
172	500.89	2.09	469.40			32687.60	
173	492.62	0.23	469.40				
174	485.64	2.67	469.40			32531.20	265.88
175	460.78	2.72	469.40				
176	442.94	3.01	469.40			39412.80	
177	447.79	2.78	469.40				
178	428.46	2.32	469.40			34095.20	231.47
179	404.79	3.82	469.40				
180	401.41	3.48	469.40			37848.80	
181	428.78	3.19	469.40				
182	423.48	0.12	469.40				
183	418.17	3.52	469.40	45356.00			
184	403.26	1.39	469.40				
185	403.68	1.27	469.40			37223.20	306.54
186	404.30	1.39	469.40				
187	416.99	1.39	469.40				
188	420.59	1.39	469.40	37223.20	6412.40	41602.40	240.86
189	378.44	1.16	469.40				
190	413.54	4.87	469.40			35972.00	
191	426.95	0.06	469.40				
192	436.77	1.80	469.40			38161.60	
193	416.10	1.39	469.40				
194	438.18	1.39	469.40			39725.60	328.44
195	426.38	1.39	469.40				
196	420.74	1.39	469.40	47903.14	4254.75	30646.11	
197	423.64	1.39	469.40				
198	409.69	1.27	469.40				
199	410.80	1.39	469.40			41654.90	
200	397.51	1.39	469.40				
201	455.68	1.04	469.40				
202	414.07	1.39	469.40			43142.58	
203	415.78	1.62	469.40				
204	417.76	1.39	469.40	36596.81			
205	401.67	1.39	469.40			37489.41	
206	381.10	1.45	469.40				
207	382.25	1.56	469.40			37191.88	
208	391.34	1.39	469.40				
209	365.97	0.75	469.40			48498.21	
210	370.21	1.56	469.40				
211	372.13	1.27	469.40	36596.81	4195.24	43440.11	
212	374.60	0.93	469.40				
213	394.10	2.09	469.40			42547.51	
214	400.05	0.93	469.40			37191.88	
215	389.25	1.39	469.40			38382.02	
216	393.60	1.56	469.40			43737.65	
217	384.55	1.39	469.40	46713.00	6605.28		
218	401.31	1.43	469.40			44332.72	
219	378.70	1.46	469.40			44630.25	291.58
220	368.58	1.52	469.40			39869.69	
221	375.39	1.41	469.40			47010.53	
222	382.73	1.41	469.40			40167.23	327.29
223	377.34	1.46	469.40			47605.60	315.39
224	373.47	1.58	469.40			42845.04	324.31

Table A.4. Continued

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
225	346.23	1.41	469.40	49390.81	3986.97	38977.09	330.26
226	356.32	1.41	469.40			38977.09	
227	364.40	1.35	469.40				
228	364.45	1.29	469.40			48498.21	360.02
229	372.30	1.41	469.40				
230	364.15	1.41	469.40				
231	387.98	1.41	469.40			40167.23	345.14
232	396.97	1.41	469.40			49093.28	
233	422.19	1.41	469.40	42249.97	6158.97	45820.39	
234	407.92	1.25	469.40			41357.37	
235	388.23	1.41	469.40				
236	385.95	1.41	469.40			41059.83	348.12
237	400.25	1.41	469.40				
238	386.27	1.41	469.40			44630.25	
239	386.77	1.41	469.40			42249.97	
240	409.26	1.41	469.40		3808.45	36001.74	
241	419.12	1.41	469.40			42249.97	312.41
242	539.23	1.23	469.40				
243	553.32	3.45	469.40				
244	545.67	0.47	469.40	45522.86		45225.32	321.34
245	530.05	1.05	469.40				
246	538.16	1.47	469.40				
247	564.37	1.47	469.40			39756.63	
248	592.05	1.83	469.40				
249	600.74	0.79	469.40				
250	600.44	0.98	469.40				
251	592.52	1.53	469.40			38239.20	243.98
252	595.33	1.47	469.40	50580.95	6843.31		
253	595.99	0.98	469.40				
254	488.02	1.53	469.40				
255	471.98	1.47	469.40				
256	474.00	1.22	469.40			35811.31	229.10
257	476.67	1.63	469.40				
258	481.61	1.34	469.40				
259	463.38	1.65	469.40				
260	451.84	1.83	469.40	47903.14	6605.28	38679.55	327.29
261	450.38	1.22	469.40				
262	449.93	1.47	469.40				
263	458.38	1.22	469.40				
264	432.70	1.47	469.40				
265	425.99	1.47	469.40			49688.35	282.66
266	413.02	1.47	469.40				
267	412.84	1.47	469.40				
268	413.95	1.71	469.40				543.24
269	406.56	1.22	469.40				
270	402.73	1.47	469.40			38084.48	288.61
271	396.37	1.47	469.40				
272	401.11	1.47	469.40				
273	388.84	1.47	469.40	42249.97			
274	388.07	1.47	469.40				
275	388.85	1.39	469.40				
276	397.27	1.51	469.40			41952.44	357.04
277	387.74	1.27	469.40				
278	397.31	1.39	469.40			40464.76	
279	389.41	1.39	469.40				
280	391.41	1.39	469.40				

Table A.4. Continued

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
281	397.41	1.39	469.40	42845.04			
282	390.01	1.39	469.40				
283	404.30	1.39	469.40				
284	396.73	1.39	469.40				
285	402.74	1.39	469.40			36299.27	261.83
286	408.67	1.39	469.40				
287	401.77	1.39	469.40	41227.20	6384.49	35501.20	
288	401.15	1.39	469.40			36360.10	
289	404.35	1.39	469.40				
290	399.91	1.39	469.40				
291	416.82	1.39	469.40			50102.50	251.94
292	405.87	1.39	469.40				
293	395.98	1.39	469.40			40368.30	
294	401.16	1.39	469.40				286.30
295	412.95	1.69	469.40				
296	410.06	1.09	469.40	42372.40	6241.34	39223.10	
297	413.37	1.39	469.40				
298	410.72	1.39	469.40			37219.00	271.99
299	421.83	1.39	469.40				
300	421.01	1.39	469.40			37505.30	
301	424.10	1.39	469.40				
302	422.58	1.39	469.40	26239.12	6025.38	34642.30	
303	401.33	1.39	402.70				
304	408.65	1.85	402.70				
305	422.81	1.23	402.70				
306	417.04	1.23	402.70				
307	392.76	1.23	402.70			24475.60	
308	387.83	1.23	402.70	23865.10	5882.15		
309	382.23	1.07	402.70				
310	375.23	1.23	402.70				
311	362.84	1.23	402.70				
312	340.11	0.92	402.70				
313	330.24	1.53	402.70	15486.22	6311.85		
314	325.99	1.23	402.70				
315	323.96	1.23	402.70				
316	311.71	1.23	402.70				
317	298.56	1.23	402.70				
318	296.61	1.23	402.70			20842.15	
319	295.59	1.23	402.70				
320	296.73	1.23	402.70	31126.80	6025.38		
321	295.68	1.23	402.70				
322	295.89	1.23	402.70				
323	300.47	1.23	402.70				
324	312.31	1.23	402.70	15486.22	4850.86		
325	306.43	1.23	402.70				
326	311.94	1.46	402.70				
327	308.80	1.46	402.70				
328	314.32	1.46	402.70			18380.78	318.61
329	316.50	1.46	402.70				
330	311.67	1.46	402.70	32523.28	4965.44		
331	313.92	1.46	402.70				
332	306.73	1.54	402.70				
333	304.34	1.37	402.70				
334	300.01	1.46	402.70				
335	292.25	1.46	402.70	20094.61	5108.68		
336	290.75	1.46	402.70				

Table A.4. Continued

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
337	292.39	1.46	402.70				
338	284.97	1.54	402.70				
339	287.26	1.37	402.70			15098.96	
340	281.70	1.62	402.70				
341	281.57	1.29	402.70	34897.30	5567.03		
342	283.47	1.46	402.70				
343	273.72	1.46	402.70				
344	273.17	1.62	402.70	28194.19	5710.27		
345	264.15	1.44	402.70				
346	257.57	1.62	402.70				
347	251.04	1.62	402.70				
348	247.62	1.76	402.70			17325.91	
349	254.50	1.62	402.70				
350	257.73	1.85	402.70	31126.80	5280.56		
351	262.75	1.44	402.70				
352	266.92	1.71	402.70				
353	268.49	1.53	402.70				
354	277.71	1.85	402.70				
355	277.34	1.44	402.70				
356	287.19	1.53	402.70				
357	290.71	1.67	402.70				
358	299.76	1.58	402.70			15802.21	211.03
359	300.56	1.62	402.70				
360	294.09	1.62	402.70				
361	259.66	1.62	402.70				
362	244.86	1.62	402.70				
363	230.52	1.62	402.70				
364	240.95	1.67	402.70				
365	247.66	1.62	402.70				
366	248.09	1.58	402.70				
367	256.88	1.58	402.70				
368	247.94	1.58	402.70			16622.66	
369	258.08	1.58	402.70	44249.71	4654.75		
370	260.72	1.58	402.70				181.72
371	267.17	1.58	402.70				
372	282.30	1.58	402.70				
373	311.69	1.58	402.70				
374	328.63	1.58	402.70				185.81
375	339.96	1.58	402.70	39330.69	4719.48		
376	330.72	1.58	402.70				
377	320.45	1.53	402.70				
378	325.89	1.62	402.70				181.72
379	324.04	0.48	402.70			17443.12	
380	318.08	1.44	402.70				
381	316.32	2.41	402.70	42307.99	4784.20		
382	328.28	1.79	402.70				184.45
383	339.64	1.01	402.70				
384	351.99	1.79	402.70	37518.42	4946.01		
385	368.52	1.58	402.70				
386	374.00	1.49	402.70				182.63
387	404.42	1.58	402.70				
388	441.52	1.79	402.70				445.16
389	456.90	1.31	402.70	29880.98	4590.03	18029.16	
390	457.61	1.88	402.70				184.45
391	460.68	1.36	402.70				
392	488.38	1.84	402.70				

Table A.4. Continued

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
393	499.67	1.49	402.70				
394	485.43	1.27	402.70				180.81
395	448.94	1.27	402.70	38683.45	4557.67		
396	437.14	2.71	402.70				
397	425.69	1.84	402.70				
398	423.69	1.62	402.70				185.36
399	386.60	1.44	402.70			29281.13	
400	345.81	2.10	402.70				
401	322.94	2.10	402.70				
402	308.68	2.10	402.70	34800.01	4654.75		183.54
403	306.25	0.70	402.70				
404	294.45	1.88	402.70				
405	290.00	1.79	402.70				
406	286.88	1.62	402.70	31175.46	4525.30		169.44
407	308.55	2.41	402.70				
408	309.49	1.53	402.70				
409	307.77	1.44	402.70				
410	308.43	1.23	402.70	22373.00	4460.58	24475.60	184.91
411	310.46	1.40	402.70				
412	316.22	1.93	402.70				
413	325.55	1.53	402.70				
414	323.04	1.53	402.70				
415	323.56	1.52	402.70				
416	321.73	1.31	402.70				
417	319.25	1.49	402.70				
418	323.99	1.75	402.70				
419	321.30	2.06	402.70				
420	318.07	2.06	402.70			20959.36	
421	310.14	2.06	402.70				
422	309.37	2.14	402.70				
423	315.01	1.88	402.70				
424	316.74	1.79	402.70				
425	324.80	1.58	402.70				
426	317.59	0.92	402.70	39119.36	5087.27		
427	312.47	1.71	402.70				
428	305.45	1.93	402.70				
429	305.04	2.10	402.70				
430	301.82	2.23	402.70			17325.91	
431	292.37	1.49	402.70				
432	290.62	1.71	402.70				
433	301.64	1.27	402.70	24843.41	5742.13		
434	300.15	1.66	402.70				
435	308.63	1.93	402.70				
436	317.13	1.36	402.70				
437	308.91	1.36	402.70				
438	339.62	2.36	402.70				
439	361.06	1.44	402.70				
440	390.06	1.40	402.70			17911.95	
441	391.58	1.62	402.70	21438.14	8689.00		
442	387.09	1.40	402.70				
443	397.27	2.14	402.70				
444	404.35	1.49	402.70				
445	393.30	0.79	402.70				
446	394.63	2.06	402.70				
447	389.09	1.58	402.70	14103.71	5611.16		
448	381.84	1.66	402.70				

Table A.4. Continued

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
449	389.70	1.31	402.70				
450	366.06	1.58	402.70			16505.46	
451	348.29	1.39	402.70				264.82
452	336.41	1.39	402.70				
453	342.11	1.27	402.70				
454	351.71	1.50	402.70	17639.95	3843.04		
455	348.72	1.31	402.70				
456	345.68	1.66	402.70				
457	358.46	1.54	402.70				
458	375.41	1.11	402.70				
459	358.22	0.83	402.70				
460	358.26	2.14	402.70	30213.26		17091.50	
461	355.65	1.43	402.70				
462	354.95	1.46	402.70				
463	354.87	1.43	402.70				
464	352.19	1.43	402.70				
465	349.07	1.43	402.70				
466	337.54	1.43	402.70				
467	345.76	1.78	402.70				
468	355.07	1.58	402.70	23402.72	3777.55		
469	355.56	1.27	402.70				
470	352.03	1.17	402.70				
471	367.52	1.20	402.70			15802.21	
472	372.41	1.01	402.70				
473	382.02	1.37	402.70				
474	385.04	1.01	402.70				
475	380.77	1.17	402.70	26284.10	2205.89		
476	388.20	1.33	402.70				
477	408.44	1.14	402.70				
478	428.85	1.30	402.70				
479	432.70	1.19	402.70				
480	411.25	1.23	402.70				
481	425.59	1.79	402.70			14981.75	
482	419.49	1.17	402.70				
483	422.07	1.43	402.70	20932.50	4048.74		729.91
484	426.12	1.43	402.70				
485	423.17	0.81	402.70				
486	432.30	1.43	402.70				
487	445.33	1.33	469.30	27495.95	3647.68		
488	447.11	0.98	469.30				
489	439.37	1.38	469.30				
490	433.16	1.38	469.30				
491	434.61	1.23	469.30			20490.53	
492	449.95	1.84	469.30				
493	438.70	1.23	469.30				
494	445.18	1.03	469.30				
495	446.71	0.81	469.30	25680.53	3647.68		
496	444.04	1.38	469.30				
497	441.49	1.38	469.30				
498	433.72	1.38	469.30				
499	430.28	1.27	469.30				
500	422.03	1.42	469.30				
501	421.35	1.61	469.30			35610.36	
502	422.97	1.07	469.30	20373.90	3619.04		
503	419.10	1.41	469.30				
504	413.38	1.46	469.30				

Table A.4. Continued

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
505	421.85	1.19	469.30				
506	427.26	1.23	469.30				
507	436.14	1.23	469.30				
508	424.63	0.73	469.30	45127.96	4868.02		
509	411.48	1.96	469.30				
510	398.22	1.86	469.30				
511	381.48	2.18	469.30			16974.29	
512	371.37	1.91	469.30				
513	357.73	2.10	469.30				824.83
514	355.57	2.10	469.30				
515	351.45	1.22	469.30	47046.67	7460.90		
516	353.82	1.22	469.30				
517	342.88	1.35	469.30				
518	329.44	1.35	469.30				
519	320.80	1.45	469.30				
520	318.47	1.32	469.30				
521	321.13	1.25	469.30				
522	332.64	1.25	469.30				
523	355.82	1.32	469.30				
524	378.20	1.32	469.30				
525	392.32	1.29	469.30				
526	390.49	2.18	469.30				
527	392.36	1.54	469.30				
528	391.78	1.75	469.30	44533.01	7467.25		
529	384.52	1.75	469.30				
530	397.65	2.44	469.30				
531	412.34	2.23	469.30				
532	415.66	0.32	469.30			22462.10	48.12
533	418.09	1.81	469.30				
534	424.62	1.70	469.30				
535	432.63	1.28	469.30	46535.88	7895.29		
536	430.74	1.13	469.30				
537	429.85	0.79	469.30				121.75
538	441.94	1.00	469.30				
539	455.14	2.08	469.30				
540	493.79	0.75	469.30				
541	522.48	1.25	469.30	43867.42	6967.13		
542	525.94	1.17	469.30	35513.98	5284.84	32157.42	100.27
543	511.21	1.44	469.30				
544	521.62	1.61	469.30				
545	519.85	1.53	469.30				
546	531.04	1.28	469.30				
547	521.63	0.58	469.30				75.73
548	539.50	1.49	469.30				
549	543.96	1.03	469.30				
550	548.32	1.69	469.30				
551	539.86	0.99	469.30				
552	516.58	2.06	469.30			29838.98	63.46
553	524.24	1.11	469.30				
554	547.57	1.90	469.30				
555	519.32	0.99	469.30				
556	531.35	2.19	469.30				
557	543.74	1.07	469.30				
558	502.79	1.40	469.30				54.26
559	510.61	2.27	469.30				
560	486.87	0.33	469.30				

Table A.4. Continued

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
561	499.47	1.16	469.30		7257.34		
562	500.08	0.41	469.30				
563	482.47	0.95	469.30			24885.93	
564	467.77	1.82	469.30				
565	442.92	1.32	469.30				
566	411.38	1.61	469.30	25588.52	4447.24		
567	424.61	1.49	469.30				
568	409.83	1.53	469.30				
569	410.91	1.49	469.30	24475.58	3789.60		
570	427.10	1.44	469.30				
571	406.42	1.44	469.30	26045.15	8054.26		
572	400.02	1.49	469.30				
573	391.61	1.49	469.30			19722.11	233.82
574	389.85	1.17	469.30				
575	390.57	0.99	469.30				
576	386.65	1.53	469.30				
577	382.43	1.40	469.30				
578	375.05	1.44	469.30	30365.90	7380.07		
579	384.73	1.57	469.30				157.61
580	375.15	0.88	469.30				
581	367.77	1.61	469.30				
582	369.48	1.00	469.30				
583	379.93	1.07	469.30				
584	390.93	1.07	469.30			13504.46	222.10
585	394.80	1.10	469.30	26677.46	6529.99		
586	396.27	1.07	469.30				
587	391.85	1.00	469.30				207.44
588	408.76	1.84	469.30				
589	425.25	1.23	469.30				
590	447.54	1.07	469.30				
591	449.00	1.07	469.30			10873.86	186.03
592	457.76	1.02	469.30				
593	462.43	1.19	469.30				
594	449.21	0.96	469.30			9126.27	195.74
595	437.01	1.50	469.30				
596	436.25	1.30	469.30				
597	432.95	0.94	469.30				
598	435.74	0.95	469.30				
599	456.69	0.94	469.30			8737.92	167.30
600	432.05	0.88	469.30	20873.92	4150.76		
601	400.54	0.96	469.30				
602	358.07	1.38	469.30				
603	317.60	1.67	469.30			8737.92	210.31
604	316.20	1.67	469.30				
605	309.35	0.87	469.30				
606	304.72	0.87	469.30			8155.39	211.70
607	310.40	1.16	469.30				
608	308.03	0.63	469.30			11860.44	118.79
609	301.72	0.63	469.30				
610	295.17	0.77	469.30				
611	291.87	0.90	469.30				
612	308.07	0.99	469.30			13848.56	222.49
613	328.60	0.39	469.30				
614	368.88	0.39	469.30				
615	424.63	0.35	469.30				
616	446.19	0.51	469.30			13380.77	228.76

Table A.4. Continued

Day	Methane Production (ml/g VS_{added})	OLR (g VS/L d^{-1})	Theoretical Max. Methane (ml/g VS_{added})	$tCOD_{influent}$ (mg/L)	$sCOD_{influent}$ (mg/L)	$tCOD_{MP}$ (mg/L)	$COD_{effluent}$ (mg/L)
617	461.78	0.45	469.30				
618	498.67	0.47	469.30				
619	498.11	0.47	469.30	26946.74	4380.28	13848.56	223.89
620	447.01	0.47	469.30				
621	410.07	0.96	469.30			14550.25	219.01
622	409.12	0.96	469.30				
623	402.39	0.58	469.30				
624	389.36	0.58	469.30				
625	383.28	0.53	469.30				
626	379.04	0.53	469.30				294.16
627	361.61	0.53	469.30			12912.98	227.37
628	346.47	0.77	469.30				
629	339.45	0.59	469.30			13324.68	229.79
630	327.70	0.59	469.30			12490.90	183.94
631	327.77	0.51	469.30				
632	359.85	1.12	469.30				
633	390.03	1.12	469.30			12729.12	224.53
634	383.72	1.12	469.30	30119.48	4520.59	13443.80	201.22
635	390.29	1.51	469.30			12683.09	228.09
636	394.88	1.50	469.30				
637	396.74	1.48	469.30			13988.26	305.21
638	390.78	1.48	469.30				
639	390.44	1.70	469.30			13276.35	323.01
640	394.02	1.71	469.30				272.79
641	374.50	1.07	469.30				
642	365.78	2.15	469.30			12463.48	277.34
643	350.36	1.61	469.30				574.58
644	333.19	1.92	469.30				
645	323.43	1.61	469.30			12577.63	394.35
646	331.57	2.91	469.30				
647	330.63	1.65	469.30				
648	309.01	1.65	469.30			12463.48	
649	290.32	2.61	469.30				
650	277.33	2.53	469.30	33924.80	7853.34		
651	275.24	2.53	469.30			15774.00	348.69
652	289.80	1.99	469.30				
653	315.30	0.81	469.30			20891.22	707.43
654	316.78	0.92	469.30			18057.12	
655	301.15	2.07	469.30				

Table A.5. Candidate adsorbent material batch test organics removal data from Chapter 4 Figure 4.4 and Figure 4.5.

Candidate Material	Dosage (g/L)	COD (mg/L)	Acetic Acid	Propionic Acid	Butyric Acid
Starting Concentration (mg/L):		*3882.84*	*3344*	*2353*	*2342*
GAC	10	3088.4176 ± 51.6	3109.9	2581.9	2266.2
	50	2699.4512 ± 17.2			
	100	2330.4788 ± 53.5	3390.7	2410.7	2145.8
	250	2104.3704 ± 36.9			
	500	576.7992 ± 178.4	2636.7	2638.9	1766.4
Purolite TANEX	10	3307.21 ± 17.2	2635.5	2016.3	1776.1
	50	3246.44 ± 343.8			
	100	2519.72	2349.6	1859.9	1556.8
	250	2448.88 ± 18.0			
	500	1396.87 ± 160.6	1615.1	1377.2	1159.9
Purolite A510	10	3173.5 ± 34.4	2667.9	2413	1783.4
	50	3003.33 ± 34.4			
	100	2507.11 ± 17.8	2478.6	2408.3	1682.1
	250	2488.66 ± 38.2			
	500	1548.26 ± 17..8	2163.1	1878.3	1407
Purolite A830	10	3367.99 ± 68.8			
	50	3453.07 ± 86.0			
	100	3238.86 ± 53.5			
	250	3000.00 ± 90.2			
	500	2999.15 ± 107.1			
Purolite A845	10	3319.37 ± 137.5			
	50	3295.06 ± 68.8			
	100	2860.37 ± 196.3			
	250	3291.01 ± 18.0			
	500	2380.94 ± 89.2			

Table A.6. Candidate adsorbent material batch test methane production from Chapter 4 Figure 4.6.

Condition	Day	Replicate	Measured Methane (ml/g COD_{added})	Average Methane (ml/g COD_{added})	Std Dev.	Cumulative Methane (ml/g COD_{added})
GAC 10 g/L	6	A	12.35	12.45	0.15	12.45
		B	12.56			
	14	A	18.34	18.59	0.35	31.04
		B	18.84			
	27	A	24.46	25.31	1.21	56.35
		B	26.16			
	40	A	26.49	25.80	0.98	82.16
		B	25.11			
	55	A	10.19	10.33	0.19	92.48
		B	10.46			
GAC 100 g/L	6	A	5.15	5.11	0.05	5.11
		B	5.08			
	14	A	12.75	12.43	0.46	17.54
		B	12.11			
	27	A	17.54	16.97	0.80	34.51
		B	16.40			
	40	A	22.72	22.29	0.60	56.80
		B	21.87			
	55	A	13.55	13.02	0.75	69.82
		B	12.50			
GAC 250 g/L	6		1.23			1.23
	14		4.47			5.70
	27		2.24			7.94
	40		6.71			14.65
	55		6.71			21.36
TANEX 10 g/L	6	A	14.30	14.17	0.19	14.17
		B	14.04			
	14	A	20.90	20.71	0.27	34.87
		B	20.51			
	27	A	36.30	35.96	0.47	70.84
		B	35.63			
	40	A	27.50	26.17	1.89	97.01
		B	24.83			
	55	A	11.00	9.82	1.67	106.82
		B	8.64			
TANEX 100 g/L	6	A	12.03	11.63	0.57	11.63
		B	11.23			
	14	A	25.26	25.11	0.22	36.74
		B	24.95			
	27	A	57.14	57.58	0.62	94.31
		B	58.01			
	40	A	14.44	15.01	0.82	109.33
		B	15.59			
	55	A	10.83	11.03	0.28	120.35
		B	11.23			
TANEX 250 g/L	6		6.56			6.56
	14		6.56			13.12
	27		13.12			26.25
	40		13.12			39.37
	55		39.37			78.74

Table A.6. Continued

A510 10 g/L	6	A	12.96	14.75	2.53	14.75
		B	16.54			
	14	A	15.73	19.09	4.75	33.84
		B	22.44			
	27	A	29.62	34.30	6.62	68.14
		B	38.98			
	40	A	23.14	23.38	0.35	91.52
		B	23.63			
	55	A	8.33	8.89	0.79	100.41
		B	9.45			
A510 100 g/L	6	A	11.20	10.54	0.93	10.54
		B	9.89			
	14	A	24.76	21.99	3.91	32.54
		B	19.23			
	27	A	47.16	45.83	1.88	78.37
		B	44.50			
	40	A	18.27	18.75	0.67	97.12
		B	19.23			
	55	A	11.79	11.11	0.96	108.23
		B	10.44			
A510 250 g/L	6		0.00			0.00
	14		6.79			6.79
	27		6.79			13.58
	40		13.58			27.16
	55		33.95			61.11

Table A.7. Methane and effluent COD data after shock-loading from Chapter 4 Figure 4.8.

Days After Shock	**Shock-load 1** *(No TANEX)*			**Shock-load 2** *(100 g/L TANEX)*			**Shock-load 3** *(No TANEX)*			**Shock-load 4** *(300 g/L TANEX)*		
	Effluent COD (mg/L)	Methane (ml/g VS_{added})	pH	Effluent COD (mg/L)	Methane (ml/g VS_{added})	pH	Effluent COD (mg/L)	Methane (ml/g VS_{added})	pH	Effluent COD (mg/L)	Methane (ml/g VS_{added})	pH
0	1244.85	299.96	5.26	1787.30	350.38	4.79	213.27	385.91	4.86	382.90	261.15	4.70
1	14128.35	270.76	5.29	14849.32	354.52	5.07	17732.03	388.15	4.85	11598.04	229.33	4.77
2	14379.96	233.09	5.33		288.78	5.16		379.43	4.88		193.20	4.84
3	14568.68	214.37	5.36		269.94		15647.80	372.94	4.85		166.82	4.91
4	12091.09	184.54	5.36		240.14			345.77		10422.34	133.25	5.02
5	11989.33	157.25	5.35		210.46	5.37		323.02	4.96		131.61	5.00
6	11684.04	139.28	7.39	9920.76	190.10	9.48	12887.61	287.45	7.40	10469.29	115.06	8.35
7	11582.27	121.51	7.40		148.56			221.19	7.40		89.17	7.52
8	13359.80	114.40			115.23		12943.94	186.80	7.41	10571.17	62.12	7.59
9	11327.86	96.31	7.38		90.11	8.29		130.50	7.41		48.03	7.73
10	10666.40	72.57	7.40	11437.24	73.22	7.52		81.38	7.41		37.70	7.41
11	12776.04	50.36	7.43		56.39	7.54		40.30		7634.11	21.18	7.41
12		39.18	7.40		41.43	7.53		14.43			22.79	8.01
13	12025.18	43.01	7.40		46.18	7.47		10.51		9149.26	29.83	7.46
14	12161.99	47.32	7.41	8088.34	53.63	7.36	12381.40	11.26			40.64	7.91
15	11479.10	47.73	7.40	7735.50	56.33	7.22		14.19		9236.14	54.78	7.61
16		51.18		7549.16	60.18	7.17		14.48			73.15	
17		53.41			62.91	7.16	12505.57	16.50		8312.09	86.94	7.79
18	10035.81	58.67	7.41	6596.80	69.50	7.22		19.00	7.44		114.89	7.56
19		62.18	7.39		91.56			20.33	7.39		144.52	7.49
20	10840.77	53.30	7.42		114.18	7.20		25.86	7.17	7748.78	169.63	7.45
21		47.38		4806.36	124.45	7.23	11317.10	36.46			207.82	7.41
22		50.73			128.55	7.35		40.57	7.41	6979.75	243.48	8.39
23	10531.17	45.32	7.44	2884.85	138.72		11358.73	50.31	7.39		261.94	
24		45.41	7.40		145.44			58.23	7.41		281.72	8.01
25		42.77	7.41		146.70		10795.68	68.30	7.41	5401.29	298.87	7.78
26	9726.21	41.87	7.41	2569.69	152.18			79.04			326.14	7.47
27		44.49	7.40		155.91	7.35		88.82		4472.92	350.33	7.41
28		46.16		1793.94	156.32	7.31		99.12			380.75	7.67
29	9788.13	48.53	7.42		178.65	7.31		109.92		4105.89	404.74	7.64
30		46.98	7.40		178.03	7.24	8520.96	120.49	7.68		403.24	
31	9855.05	44.25	7.41	1486.89	170.14	7.24		140.82			400.35	
32		54.34	7.42		164.41	7.24	6786.77	158.94	7.67		401.83	7.57
33		63.06			175.00	7.20		176.35	7.64	3628.20	386.59	7.55
34	7930.82	72.52	7.41	965.68	177.91	7.17		199.37		3675.24	365.28	7.45
35		76.41	7.42		182.05	7.16	5069.19	212.63	7.47		373.72	
36		81.29			189.53	7.18		226.84	7.40	3440.07	374.67	
37	6875.60	88.40	7.41	917.20	195.26	7.16	3637.55	194.12	7.41		361.89	
38		94.77	7.42		193.82	7.22		196.74			344.88	
39		98.82	7.43		197.74			199.16	7.42	3202.13	331.72	
40	6130.73	117.51	7.42	917.20	207.23	7.22		205.92		3054.36	298.15	
41		121.15			198.68			213.17		3177.51	299.82	
42		129.30		892.95	209.11	7.21		220.39	7.38		298.22	
43	5392.89	138.55	7.41		224.82	7.24	3221.91	213.26	7.40	2931.22	303.37	
44	5711.36	133.00	7.40		233.65			208.03			308.80	
45	5074.42	128.67	7.39	478.06	236.49	7.29		202.55			313.70	
46		123.52	7.40		240.88			198.41		2368.57	323.46	
47	4742.37	127.45	7.41		245.91	7.34	1767.18	196.17	7.39		318.91	
48		126.94	7.38	612.01	244.88	7.28		185.51		2179.49	316.67	
49	4615.83	123.46	7.40				2205.91	233.55	7.40		336.80	
50		122.84	7.42					237.07	7.33		348.03	
51	4463.98	131.08	7.41					234.95	7.42	1659.52	350.39	
52		116.97	7.40					234.09			372.19	
53	4084.36	116.57	7.40					231.77	7.42	811.37	376.19	
54		121.48	7.35					244.51			384.37	
55	3837.93	120.77	7.45					264.51		1243.03	385.53	
56		132.06	7.48					273.00	7.31		383.40	
57		136.61	7.40					274.50		1578.76	389.81	7.63

Table A.7. Continued

Days After Shock	**Shock-load 1** *(No TANEX)* Effluent COD (mg/L)	Methane (ml/g VS_{added})	pH	**Shock-load 2** *(100 g/L TANEX)* Effluent COD (mg/L)	Methane (ml/g VS_{added})	pH	**Shock-load 3** *(No TANEX)* Effluent COD (mg/L)	Methane (ml/g VS_{added})	pH	**Shock-load 4** *(300 g/L TANEX)* Effluent COD (mg/L)	Methane (ml/g VS_{added})	pH
58	3400.27	144.96	7.33				2348.62	278.78	7.33			
59		151.04						269.57	7.32			
60	3521.84	160.50	7.43				2308.58	268.81	7.31			
61		175.63	7.42					281.38				
62	3906.57	191.43	7.45					298.86	7.17			
63		191.72					1542.95	304.13	7.40			
64	3422.60	200.92	7.41					299.98	7.40			
65	3218.83	218.75	7.45					286.76	7.39			

Table A.8. Continuous fermentation VFA data from Chapter 5 Figure 5.4.

HRT (day)	Day	Influent (mg/L)	Fermenter Effluent (mg/L)	HRT (day)	Day	Influent (mg/L)	Fermenter Effluent (mg/L)
	1		5289.57		100		7864.29
	3		4801.42		101	4778.65	
	4		7497.54		114		10064.10
Start-up	6		4774.16		115	7604.12	
	7		6658.15		119		11386.33
	9		9087.89		120		10665.45
	15	2966.30	9593.17		122	7813.38	10915.10
	21		9774.93		126		9913.28
	23		9231.45		127		12176.68
	28		9829.34		128		12552.79
	31		9178.68		132		13801.64
	34	2829.11			133		13033.28
	35		8484.78		134		13263.12
	37		8573.45		135		12983.97
	38		8913.94		135		12983.97
2.50	41		7225.79	3.50	138		7328.70
	42		8006.10		139		9434.20
	43		8942.98		141		9150.31
	45		10753.58		142		9753.52
	48	3990.40	9020.16		143		11580.67
	50		8481.67		146		8490.12
	52		8561.73		149		9740.64
	55		9155.91		160		6348.37
	57	2897.70			162		9773.48
	58		8698.79		164		9870.73
	62	3444.05	9024.06		168		8215.37
	65		9650.33		170		9461.56
	66		8661.49		171		10996.30
	69		8605.07		174		10463.33
	72		9302.08		175		11065.61
3.00	77		9497.42		177		10314.64
	79		7563.85				
	83	3170.88	8037.60				
	90		12157.84				
	91		12622.62				
	92		9265.05				
	94		9635.95				
	98		8811.15				

Table A.9. Biomass selection phase TSS, VSS, and PHA data from Chapter 5 Figure 5.5.

Day	TSS	VSS	PHA Content (g/g VSS)
0	2.37	1.32	
2			2.47
3	1.35	1.25	
7	2.78	2.58	
9	3.50	3.11	
10			1.52
14	2.67	2.26	
21			2.40
22	2.74	2.39	
29	2.30	2.22	
36	2.92	2.68	
43			1.22
44	2.82	2.53	
49			1.67
50	2.97	2.88	
56			1.10
57	2.40	2.27	
64	2.31	2.13	
70			8.46
78	2.27	2.12	
85	2.42	2.15	
86			2.30
99	2.10	1.77	
120	2.03	1.72	13.85
127	2.43	2.02	
140	3.55	2.99	17.57

Table A.10. Baseline condition life cycle impact assessment results from Chapter 6 Figure 6.3.

AnMBR	Amount	Cost	Ecotoxicity	Acidification	HH Carcinogenics	Ozone depletion	Fossil fuel depletion	Respiratory effects	HH Non carcinogenics	Smog	Global warming	Eutrophication
	per m³	*$*	*CTUe*	*kg SO2 eq*	*CTUh*	*kg CFC-11 eq*	*MJ surplus*	*kg PM2.5 eq*	*CTUh*	*kg O3 eq*	*kg CO2 eq*	*kg N eq*
Electricity (kWh)	24.8827	2.5132	0.0547597	0.04726821	9.43239E-10	7.894E-07	9.59960792	0.003416149	-2.2781E-08	0.323259	12.66865	0.001118727
PACl (kg)	0.01429	0.0036	2.484E-05	11.6781518	3.02671E-08	2.577E-13	0	0	0.000732328	0.128572	0.000316	0.00569444
Waste landfilling (kg)	3.28571	0.1643	0.0001543	0.00427143	1.96282E-11	1.061E-08	0.42931143	4.3034E-05	8.85612E-12	0.017776	1.684914	0.003417143
Transport (kg)	3.28571	0.0007	3.909E-06	2.6943E-05	1.46631E-14	9.532E-12	0.0087262	1.13979E-06	5.83048E-15	0.000597	0.00432	1.60495E-06
NaOCl (L)	1.2E-08	3E-09	3.897E-07	1.9737E-10	1.8692E-15	1.427E-14	2.9282E-08	2.84746E-11	1.3054E-14	2.05E-09	2.92E-08	1.45981E-10
Citric Acid (L)	3.3E-09	5E-10	8.506E-07	7.6772E-10	4.32712E-15	2.09E-14	9.9866E-08	8.73542E-11	2.29679E-14	6.99E-09	1.05E-07	4.32634E-10
Dissolved Methane (kg)	0.00175	0	0	0	0	0	0	0	0	0	0.147391	0
Recovered Methane (kWh)	-47.792	-4.827	-0.105176	-0.09078794	-1.81168E-09	-1.52E-06	-18.437942	-0.00656139	4.37552E-08	-0.62088	-24.3326	-0.002148736
	Total	-2.145	-0.050232	11.6389304	2.94183E-08	-7.16E-07	-8.4002968	-0.00310107	0.000732349	-0.15068	-9.82705	0.008083179

PHA	Amount	Cost	Ecotoxicity	Acidification	HH Carcinogenics	Ozone depletion	Fossil fuel depletion	Respiratory effects	HH Non carcinogenics	Smog	Global warming	Eutrophication
	per m³	*$*	*CTUe*	*kg SO2 eq*	*CTUh*	*kg CFC-11 eq*	*MJ surplus*	*kg PM2.5 eq*	*CTUh*	*kg O3 eq*	*kg CO2 eq*	*kg N eq*
Electricity (kWh)	0.05825	0.0059	0.0001282	0.00011066	2.20826E-12	1.848E-09	0.02247412	7.99772E-06	-5.3334E-11	0.000757	0.029659	2.61911E-06
PACl (kg)	7.4E-05	2E-05	1.279E-07	0.06015033	1.55896E-10	1.328E-15	0	0	3.77198E-06	0.000662	1.63E-06	2.93302E-05
Polypropylene (kg)	-0.0036	-0.014	-6.976E-05	-2.2238E-05	-4.74696E-17	0	-0.0341182	-1.4125E-06	-1.051E-15	-0.0002	-0.00712	-5.3241E-06
Polystyrene (kg)	-0.0036	-0.014	-0.0001863	-3.9261E-05	-9.63304E-14	0	-0.0418077	-2.4991E-06	-2.0454E-14	-0.00031	-0.01224	-4.67217E-06
Waste landfilling (kg)	0.01244	0.0006	5.84E-07	1.6166E-05	7.42853E-14	4.017E-11	0.00162478	1.62867E-07	3.3517E-14	6.73E-05	0.006377	1.29326E-05
Transport (kg)	0.01244	3E-06	1.479E-08	1.0197E-07	5.54942E-17	3.607E-14	3.3025E-05	4.31369E-09	2.20662E-17	2.26E-06	1.63E-05	6.07411E-09
NaOCl (L)	1.2E-11	3E-12	3.897E-10	1.9737E-13	1.8692E-18	1.427E-17	2.9282E-11	2.84746E-14	1.3054E-17	2.05E-12	2.92E-11	1.45981E-13
Citric Acid (L)	3.3E-12	5E-13	8.506E-10	7.6772E-13	4.32712E-18	2.09E-17	9.9866E-11	8.73542E-14	2.29679E-17	6.99E-12	1.05E-10	4.32634E-13
Dissolved Methane (kg)	3.3E-06	0	0	0	0	0	0	0	0	0	0.000279	0
Recovered Methane (kWh)	-0.0265	-0.003	-5.822E-05	-5.0258E-05	-1.00289E-12	-8.39E-10	-0.0102067	-3.6322E-06	2.42216E-11	-0.00034	-0.01347	-1.18948E-06
	Total (PS)	-0.01	-0.000115	0.06018774	1.57079E-10	1.049E-09	-0.0278825	2.03363E-06	3.77195E-06	0.000835	0.010623	3.90263E-05
	Total (PP)	-0.01	9.49E-07	0.06020477	1.57175E-10	1.049E-09	-0.0201930	3.12018E-06	3.77195E-06	0.000942	0.015743	3.83744E-05